UNIVERSITY OF ALBERTA

EFFECTS OF AN ACUTE BOUT OF EXERCISE ON NEUTROPHIL COUNT AND FUNCTION, SALIVARY IgA AND CORTISOL IN CHILDREN AND ADOLESCENTS RECEIVING MAINTENANCE THERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA

BY

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

FACULTY OF PHYSICAL EDUCATION AND RECREATION

EDMONTON, ALBERTA
Spring 2005

0-494-08105-8

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DEDICATION

To my mother for her endless support, encouragement and love in every path of life I have chosen. You are a phenomenal woman, mother and friend. To my father for his humour and positive outlook on life even in the most challenging of times and for always wanting and providing the very best for his children. "Of life's two chief prizes, beauty and truth, I found the first in a loving heart and the second in a labourer's hand."

~ Khalil Gibran ~

ABSTRACT

This was a nonrandomized controlled trial to determine the effects of acute exercise on neutrophil count and function, salivary cortisol and IgA in children and adolescents receiving maintenance treatment for acute lymphoblastic leukemia (ALL). Ten males participated in the study, four ALL patients matched with six healthy controls. A significantly reduced VO_{2peak} was found in the ALL participants who also reported 276 less exercise minutes per week than the controls. A significant increase in absolute neutrophil count from pre to post-exercise was observed in each group. Neutrophil function was significantly depressed in the ALL group at the basal level, however increased in both groups following exercise and stimulation. No statistically significant differences were found in salivary cortisol or IgA, however similar increases were demonstrated following exercise in both groups. This pilot study suggests that 30 minutes of moderate intensity exercise does not cause an adverse immune response in ALL participants.

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Kerry Courneya, for his guidance, support and words of encouragement throughout this project. His knowledge, work ethic and dedication to the advancement of this area of research are truly inspiring. Thank you for the many opportunities you have provided me with.

A very sincere thanks to my committee members, Dr. Catherine Field, Dr. Gordon Bell and Dr. Paul Grundy for their advice, expertise and assistance with this thesis. Thank you for your genuine interest and support in my research.

I would like to express my appreciation to all of the nurses and staff in the Pediatric Oncology Outpatient Unit, 4E2 at the Stollery Children's Hospital, especially, Monica Whitehead and Diane Arndt for their assistance in this study.

I would also like to thank Sue Goruk and the Human Nutrition lab for all their help with this project and answering my many questions.

Thank you to my lab mates and colleagues for their assistance with this thesis. You are a great bunch and it has truly been a pleasure working with all of you.

To my sisters, Alishan and Azra, thanks for your love, support, humour and encouragement, and thanks for always remembering.

To my girls, thank you for your continuous encouragement, interest and enthusiasm in my research. I could always come to each of you to share my exciting news. Thank you for your friendships, for many good times and laughs and for always making me feel like I had family here.

And finally, a very big thank you to all the participants and their parents for partaking in this study. This thesis would not have been possible without you.

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LIST OF ABBREVIATIONS

1. ALL – acute lymphoblastic leukemia
2. AML – acute myeloid leukemia
3. PYLL – potential years of life lost
4. WBC – white blood cell
5. RBC – red blood cell
6. ANLL – acute nonlymphocytic leukemia
7. CML – chronic myelogenous leukemia
8. CNS – central nervous system
9. CSF – cerebrospinal fluid
10. QOL – quality of life
11. PCP – pneumocystis cainii pneumonitis
12. ANC – absolute neutrophil count
13. sIgA – secretory immunoglobulin A
14. IFN – interferon
15. IL – interleukin
16. CSF – colony stimulating factors
17. TNF – tumor necrosis factors
18. NK – natural killer (cells)
19. ROS – reactive oxygen species
20. MHC – major histocompatibility complex
21. MALT – mucosal-associated lymphatic tissue
22. GALT – gut-associated lymphoid tissue

23. BALT – bronchus-associated lymphoid tissue

24. NALT – nasal-associated lymphoid tissue

25. URTI – upper respiratory tract infection

26. LTPA – leisure time physical activity

27. HREB – Health Research Ethics Board

28. NAPP – Northern Alberta Pediatric Program

29. SCH – Stollery Children's Hospital

30. CCI – Cross Cancer Institute

31. BMI – body mass index

32. ECG – electrocardiogram

33. HRmax – maximal heart rate

34. RERmax – maximal respiratory exchange ratio

35. AT – anaerobic threshold

36. EDTA – ethylenediamine tetraacetic acid

37. CBC – complete blood count

38. DHR – dihydrorhodamine 123

39. PMA – phorbol myristate acetate

40. NSB – non-specific binding

41. TMB – tetramethylbenzidine

42. ANOVA – analysis of variance

43. HGB – hemoglobin

44. HCT – hematocrit

45. Neut % – relative neutrophil percent

46. LY % – relative lymphocyte percent

47. ALC – absolute lymphocyte count

48. Eos % – relative eosinophil percent

49. CDC – Centres for Disease Control and Prevention

50. ACSM – American College of Sports Medicine

51. WHO – World Health Organization

I: CHAPTER ONE

INTRODUCTION

I – 1. INTRODUCTION

Although childhood cancers are rare, an estimated 9,200 new cases were expected to occur among children 0-14 in 2004 in the U.S.[5] From 1996-2000, cancer was diagnosed in an average of 1,289 Canadian children every year, and 231 died each year from the disease.[2] In Canada, leukemia accounted for 26% of all new cases and 30% of deaths due to cancer in children, and remained the most common cancer among children and adolescents.[2] According to the American Cancer Society, leukemia accounted for 30% of cases in children 0-14 years and 25% of cancers occurring before age 20.[8] In addition, the American Cancer Society predicted approximately 2,860 children were diagnosed with leukemia in the United States during the year 2004.[5] Of these, 2,230 were diagnosed with acute lymphoblastic leukemia (ALL) and many of the remaining diagnosed with acute myeloid leukemia (AML).[5] ALL is most common in early childhood peaking between ages 2 and 3 years of age while AML is most common during the first 2 years of life or among adults and is less common among older children.[8] Over 50 years ago, ALL was a universally fatal disease in that half of the affected children succumbed within four months of diagnosis.[80] Currently, approximately 80% of children with acute lymphoblastic leukemia are alive five years after diagnosis.[127] Improved survival has been dramatic for acute lymphocytic leukemia as well as lymphomas and kidney cancer.[2] Generally, cancer is the chief cause of death by disease in children between the ages of 1 and 14.[4] Among children aged 0-19, cancer ranked as the sixth leading cause of potential years of life lost (PYLL) after perinatal causes, congenital anomalies, motor vehicle accidents, other accidents and suicide.[3] The PYLL due to cancer deaths in children aged 0-19 in Canada in 2000 was 15,000 years.[2] Major advances have occurred in the detection and treatment of childhood cancers, which have resulted in a 50% decrease in mortality rates from the early 1950's.[5] Improvements in childhood cancer survival reflect shifts towards multi-disciplinary care improving overall outcomes and decreasing morbidities from complications of the malignancy and/or its treatments.

I – 1a. Leukemia

Leukemia is a cancer of the white blood cells (WBCs) known as leukocytes. It is characterized by an increase in the number of abnormal leukocytes. The basic abnormality of leukemia lies in the blood forming tissues, bone marrow, lymph nodes and spreads to the blood, spleen, liver, central nervous system, and other organs. There is no tumor; instead, immature cells proliferate and escape normal control mechanisms, remaining young, thereby negatively affecting bone marrow function. Overabundances of abnormal cells displace the healthy blood cells in the bone marrow. Crowding of healthy red blood cells (RBCs) and platelets force immature WBCs, called blasts out into the bloodstream. As abnormal cells continue to divide, the normal blood cell production decreases leaving the patient anemic and susceptible to infections and bleeding.

Leukemia is classified by the speed with which the disease develops and the predominant type of cell present in the bone marrow and blood. *Acute* leukemias develop rapidly, populating the blood and bone marrow with immature cells, and occur most frequently in the young. Acute leukemia is divided into acute lymphocytic or lymphoblastic leukemia and acute nonlymphocytic leukemia (ANLL) also known as acute myeloid leukemia. AML is the acute form that occurs more often in adults. In contrast, *chronic* leukemias progress at a slower pace, produce greater numbers of mature white blood cells, and also occur mostly in adults. Chronic myelogoenous leukemia (CML) is extremely rare in children, accounting for only 2% of leukemias.[8] Thus, for the purposes of this paper, ALL will be discussed further in detail.

I – 1b. Acute Lymphoblastic Leukemia

Acute lympoblastic leukemia is a cancer of the lymphocyte-forming cells called lymphoblasts. It is divided into three major categories: L1, L2, or L3 based predominantly on morphology and immunologic type (B-cell or T-cell). L1 lymphoblasts are most common in children and are smaller cells. L2 are larger cells and account for approximately 10% of ALL cases.[8] The L3 lymphoblast is the rarest subtype. **Table I - 1** shows ALL type by frequency. About 85% of ALL is B-cell ALL and the most common subtype of B-cell ALL is early precursor B or early pre-B.[8] The pre-B ALL subtype accounts for 20 – 25% while mature B-cell leukemia accounts for 2 – 3% of childhood ALL and has the L3 morphology.[8] Approximately 13 – 15% of ALL is the T-cell type.[8] This type of leukemia affects boys more than girls and generally affects children at an older age than the B-cell subtype. It is often associated with an enlarged thymus and early spread to the spinal fluid.

Table I – 1. ALL Type by Frequency[8]

Type	Frequency
Early Pre-B	57 – 65%
Pre-B	20 – 25%
Transitional pre-B	2 – 3%
B-Cell	2 – 3%
T-Cell	13 – 15%

I – 1c. Treatment of Acute Lymphoblastic Leukemia

When leukemias are first diagnosed, there are approximately one trillion leukemia cells in the body. Elimination of 99% of these cells is enough to achieve remission; however, this still leaves about 10 billion leukemia cells in the body, which must also be eliminated. Thus, treatment of children with ALL is divided into three phases: induction, consolidation or intensification, and maintenance. Most children with ALL require treatment lasting between two and three years.[80] Refer to **Table I - 2** for a brief summary of the treatments by phase.

I – 1c – i. Induction

Children with ALL are divided into standard-risk, high-risk, and very high-risk groups to ensure correct types and doses of drugs are administered. The goal of the first phase of therapy is to achieve complete remission defined as the absence of clinical signs of disease, reduction of leukemic cell numbers to an undetectable level (bone marrow less than 5% lymphoblasts and normal cellularity), and restoration of normal hematopoiesis as evidenced by normal peripheral blood counts.[90] More than 95% of children with ALL enter remission following one month of treatment and fewer than 3% of children die of complications during this initial treatment.[8]

Children with standard-risk ALL usually receive three drugs, namely, prednisone, asparaginase, and vincristine for the first month of treatment.[8] A fourth drug, an anthracycline (most often daunomycin) is added for high-risk children.[8] All children receive central nervous system (CNS) preventative therapy, which consists of intermittent intrathecal methotrexate or intrathecal triple therapy (methotrexate, cytarabine, and hydrocortisone).[80] Standard CNS prophylaxis occurs twice during the first month and 4 to

6 times during the next month or two months. This type of therapy involves spinal taps to instill chemotherapy into the cerebrospinal fluid (CSF) in order to destroy any leukemia cells that may have spread to the central nervous system. Intrathecal chemotherapy is repeated less often during consolidation and maintenance. Children with high-risk disease often receive prophylactic cranial radiation at doses of 1,200 to 1,800 cGy in addition to standard prophylactic intrathecal chemotherapy.[80] Those patients with leukemia detected in the CSF when diagnosed will need to receive therapeutic doses (2,400 cGy) of craniospinal radiation therapy in addition to the intrathecal therapy.

I – 1c – ii. Consolidation or Intensification

Treatment must continue following remission induction, as relapse is inevitable due to a significant number of undetectable leukemic cells remaining.[80] This intensive phase of chemotherapy lasts four to eight months. It is important to reduce the number of leukemia cells still remaining in the body from 10 billion to none. Children with standard-risk ALL are usually treated with intermediate dose levels of methotrexate followed by leucovorin rescue and 6 mercaptopurine. These drugs, which are usually given orally, are called antimetabolites. Children demonstrating high-risk ALL will receive chemotherapy in higher doses and may receive other drugs in addition.

I – 1c – iii. Maintenance

Maintenance therapy is usually antimetabolite-based with daily administration of mercaptopurine and weekly doses of methotrexate. Intermittent pulses of prednisone (given by mouth) and vincristine (given intravenously) are also included in most maintenance regimens and are associated with reduced incidence of relapse.[9] The latter two drugs are given for brief periods every four to eight weeks.

Despite the current risk-directed therapy, approximately 20% of children will sustain a relapse of their disease, with the most likely etiology being development of drug resistance.[132] Children relapsing while on therapy or within six months of discontinuation of therapy have only a 10% chance of three-year survival with conventional chemotherapy regimens.[80] Thus, according to Landier (2001), allogeneic stem cell transplantation following chemotherapy with or without total body irradiation is the treatment choice for these patients. The potential for long-term survival following the transplantation is estimated to be 40-50%.[121] In addition, allogeneic stem cell transplantation may be recommended in first remission for certain children with very high-risk features at diagnosis.

Table I – 2. Treatment for Acute Lymphoblastic Leukemia [6, 7, 80]

Treatment	Description
Induction Phase	
Prednisone, asparaginase, vincristine	All 3 drugs given for the first month of treatment to induce remission and restore normal hematopoiesis.
Anthracycline	Added to the combination for high-risk children.
Intrathecal chemotherapy	In all phases for all patients to eradicate subclinical CNS leukemia; prophylactic cranial radiation for high risk patients usually during consolidation
Consolidation Phase	
Methotrexate, leucovorin rescue, 6 mercaptopurine	These antimetabolites, methotrexate intraveneously and mercaptopurine orally are given for 4 to 8 months of therapy to strengthen remission and direct treatment to CNS sanctuary sites.
Maintenance Phase	
antimetabolite drugs, vincristine (IV) and prednisone (mouth)	Treatment in this phase is 2 years to maintain remission.

I – 1d. Side Effects of Cancer Treatment

The impact of a cancer diagnosis and its associated treatments in children often cause numerous negative side effects that greatly affect their quality of life (QOL). Children are often very symptomatic and highly distressed by their physical and psychological symptoms.[37] Lack of energy, pain, nausea, difficulty swallowing, mouth sores, and insomnia are frequent complaints of children aged 10-18 using the Memorial Symptom Assessment Scale.[37] A similar survey conducted with younger children aged 7-12 reported lethargy, pain, insomnia, itch, worry, nausea, and sadness as highly distressing symptoms.[38] Further, many adolescents display an increased tendency towards problems with peer relationships,[117] depression,[50, 76, 81, 168] and altered perceptions of self and body image.[80, 122] Compounding these psychosocial concerns, anticancer therapies may also cause nausea and vomiting[6, 54, 56, 64, 80], loss of appetite[37, 56], headaches[6], temporary hair loss[6, 56], mucositis and stomatitis (mouth and throat sores)[6, 15, 54, 56], fatigue[6, 42, 43, 56, 120], anemia[6, 54, 56, 80], changes in the menstrual cycle[6], and damage to the ovaries or testicles that may result in infertility.[6]

Functional side effects in children undergoing cancer treatment such as decreased exercise tolerance/work load,[135] reductions in total daily energy expenditure,[158] reduced cardiovascular and pulmonary function,[72] impaired muscle strength,[65] increased risk of obesity[150] and excessive weight gain related to glucocorticoid therapy[80] are reported as well as an increased risk of osteopenia due to the disease and intensive chemotherapy.[154] Bone mineral density is significantly reduced at diagnosis and remains low during therapy resulting in fracture rates six times higher in ALL patients than healthy controls.[154] Osteopenia/osteoporosis has been observed throughout the post-treatment period for as long as 20 years resulting in musculoskeletal pain, disturbed gait, kyphosis, lordosis and growth failure.[60]

Some long term survivors of ALL in childhood have been found to have anthracycline induced cardiomyopathy that can cause reduced exercise capacity,[73] axonal damage throughout the nervous system, and demyelination within the spinal cord.[61] Limitations in running speed, balance, strength and flexibility,[165] reduced levels of energy expenditure, and physical activity have also been reported.[158]

In a child with acute lymphoblastic leukemia, there is concern of immunosuppression due to chemotherapy treatments, which may further lead to an increase in susceptibility to infection, [6, 54, 56, 111, 139, 155] slow reconstitution of immune function after treatment and may enhance the risk of cancer reoccurrence.[14] There is potential for electrolyte imbalance, renal compromise related to lysis of lymphoblasts, leukopenia, anemia,[6] thrombocytopenia and infection due to fever and/or neutropenia.[54, 56, 80] Infectious complications of cancer treatment are potentially life threatening and remain the leading cause of morbidity and mortality in children with cancer.[33, 54] In a retrospective study conducted with 14 pediatric patients undergoing BMT, it was found that all 14 patients developed infectious complications following transplantation with a total of 24 bacterial infections among 11 patients, 21 fungal infections among 11 patients, 2 parasitic infections, and 4 viral infections.[139] In addition, Groll and colleagues reported that pneumocystis carinii pneumonitis (PCP) is one of the most opportunistic infections in children and adolescents with cancer.[59] Patients receiving chemotherapy have a higher incidence of P carinii infection.[54] Its high frequency and considerable mortality have led to primary chemoprophylaxis in immunosuppressed children.[54, 59]

Myelosuppression associated with chemotherapy [30, 33, 35, 54, 56, 155] manifests as early as one week after systemic therapy and may continue up to one month after therapy is complete, depending on the regimen administered.[54] Differing degrees of myelosuppression may occur in response to the effects of anticancer agents on the bone marrow.[56] **Table I – 3** shows the grading criteria for myelosuppression from expected to toxic levels.

Table I – 3. Toxicity Criteria of Myelosuppression[56]

Myelosuppression	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia					
Leukocytes (WBC) -life span 12 h (/mm^{3})	>4,000	3,000-3,900	2,000-2,900	1,000-1,900	<1,000
Granulocytes (/mm^{3})	>2,000	1,500-1,900	1,000-1,400	500-900/mm	<500
Thrombocytopenia					
Platelets -life span 10 days (/mm^{3})	100,000	75,000-99,000	50,000-74,000	25,000-49,000	25,000
Hemorrhage	none	petechiae	mild blood loss	gross blood loss	debilitating loss
Anemia					
Erythrocytes (RBC) -life span 100-120 d (g/100mL)	11	9.5-10.9	8.0-9.4	6.5-7.9	<6.5

Although reports are inconsistent, generally, anticancer therapies cause depletions in numerous immunologic variables. Neutropenia is one of the most common and potentially serious complications of therapy for malignancy.[30, 33, 54, 58, 110, 155] A low absolute neutrophil count (ANC) with fever can put the young patient at high risk for a serious bacterial infection[15, 35, 155] or possibly bacterial or fungal sepsis.[33, 35, 54, 58, 111, 119, 155] ANC is calculated by multiplying the percentage of neutrophils (segmented neutrophils plus bands) by the total white blood cell count.[54] The threshold for defining neutropenia is an ANC<1000/L; moderate neutropenia is an ANC<500/L and severe neutropenia is an ANC<200/L.[54]

A cancer diagnosis is a traumatic experience for the child as well as the child's parents. Parents often demonstrate high anxiety levels,[19, 133] post-traumatic stress symptoms,[19, 133] and become increasingly overprotective.[69] The possibility of a severe and life-threatening illness causes everyday concern in their child's diet or appropriate discipline.[167] Parents may limit the amount of activity or play time due to the disease and/or its treatments. Parents may also believe that their child is physically vulnerable and unable to resume daily activities such as schooling or physical activity. One-third of parents who considered their child vulnerable were also considered overprotective in a study conducted by Thomasgard and Metz.[147] The study determined a statistically significant discrepancy in that parents reported their children to experience more limitations in their lives than did the children themselves.[83] This overprotectiveness and the limitations imposed by parents may have implications for the child including peer relationships, social well-being, self-esteem and overall quality of life.

Clearly, appropriate supportive interventions are needed to address the physical sequelae, functional side effects, quality of life and immunosuppression in children receiving treatment for cancer in order to prevent further complications and morbidities.

I – 2. STATEMENT OF THE PROBLEM

As stated previously, infectious complications are the leading cause of morbidity and mortality in children diagnosed with cancer. At present, there is no known intervention to address the broad array of immunosuppressive side effects including neutropenia caused by anticancer treatments. Physical exercise may be one intervention that can influence the immune system as well as address a range of QOL issues, which entails not only emotional, psychological and social well-being, but also physical and functional well-being.[40] Moreover, a strong theoretical basis for the use of exercise as a quality of life intervention in cancer survivors is discussed by Hicks.[63] Unfortunately, there is a paucity of research in this area in childhood cancer patients. It is unknown whether physical exercise is beneficial or detrimental to the child's immune system, whether it will cause increased susceptibility to infection during cancer treatment. Oncologists do not encourage or discourage exercise in children with ALL, as the role of exercise during cancer therapy is not well understood.

The purpose of this exploratory, pilot study was to determine the effects of an acute bout of moderate intensity exercise on neutrophil count and function, salivary immunoglobulin A and cortisol in children and adolescents undergoing maintenance therapy for acute lymphoblastic leukemia and to explore if exercise-induced immune responses differ for ALL patients versus healthy age- and gender-matched control individuals.

I – 3. HYPOTHESIS

1. An increase in the absolute count and function of neutrophils in peripheral blood will occur immediately following the acute bout of exercise.
2. An increase in salivary cortisol will occur from pre-exercise to post-exercise and will continue to increase at 1-hour and 2-hours post exercise, which will coincide with the concentration of neutrophils into circulation at 2-hours.
3. A decrease in salivary IgA is not expected to occur following the exercise intervention, 1-hour or 2-hours post intervention.
4. A different immune response is expected to be observed in the ALL patients versus healthy, age- and gender-matched control participants.

II: CHAPTER TWO

LITERATURE REVIEW

Knowledge of the immune system is important in understanding its role in the recovery from cancer treatments and the rationale for current therapeutic approaches such as physical exercise in improving immunity. The literature has been reviewed in the following sections: 1.) The Immune System, 2.) Exercise Training and the Immune System, 2a.) Acute Exercise and Salivary IgA, 2b.) Acute Exercise and Salivary Cortisol, 2c.) Acute Exercise and Neutrophils, 3) Pediatric Cancer and Exercise, 4.) Summary.

II – 1. THE IMMUNE SYSTEM

The immune system protects the body against pathogens that can cause infection and/or disease such as viruses, bacteria, fungi, and multicellular parasites. It is comprised of two functional components that work together in a co-ordinated manner. The innate immune system provides a first line of defence against any foreign material while the acquired immune response is activated.[48] The innate division encompasses soluble and cellular factors, physical barriers, and the reticuloendothelial system.[55] The acquired or adaptive immune system includes cellular and soluble components. It produces an antigen-specific response and retains immunologic memory of each pathogen, which stimulates increasingly effective defence mechanisms.[11, 82, 130] Both systems are illustrated in **Figures II – 1 and II – 2** below.

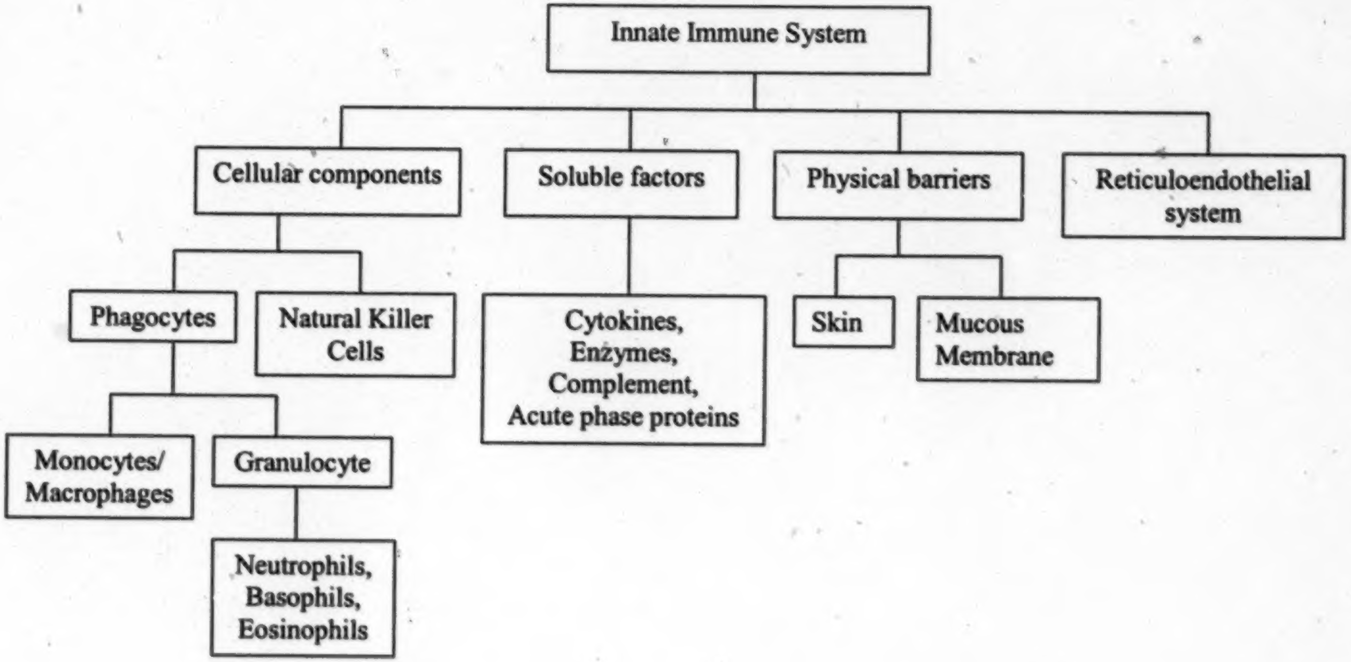
Innate Immune System
Cellular components
Soluble factors
Physical barriers
Reticuloendothelial system
Phagocytes
Natural Killer Cells
Cytokines, Enzymes, Complement, Acute phase proteins
Skin
Mucous Membrane
Monocytes/ Macrophages
Granulocyte
Neutrophils, Basophils, Eosinophils

Figure II - 1. Innate Immune System

Integrated from Roitt, I. *Immunology*. 5th Edition ed. London: Mosby International Ltd., 1998, 423.,
Leffell, M., A. Donnenberg, and N. Rose. *Handbook of Human Immunology*. New York: CRC Press, 1997, 640.,
Goldsby, R., T. Kindt, and B. Osborne. *Kuby Immunology*. New York: W.H. Freeman, 2000.

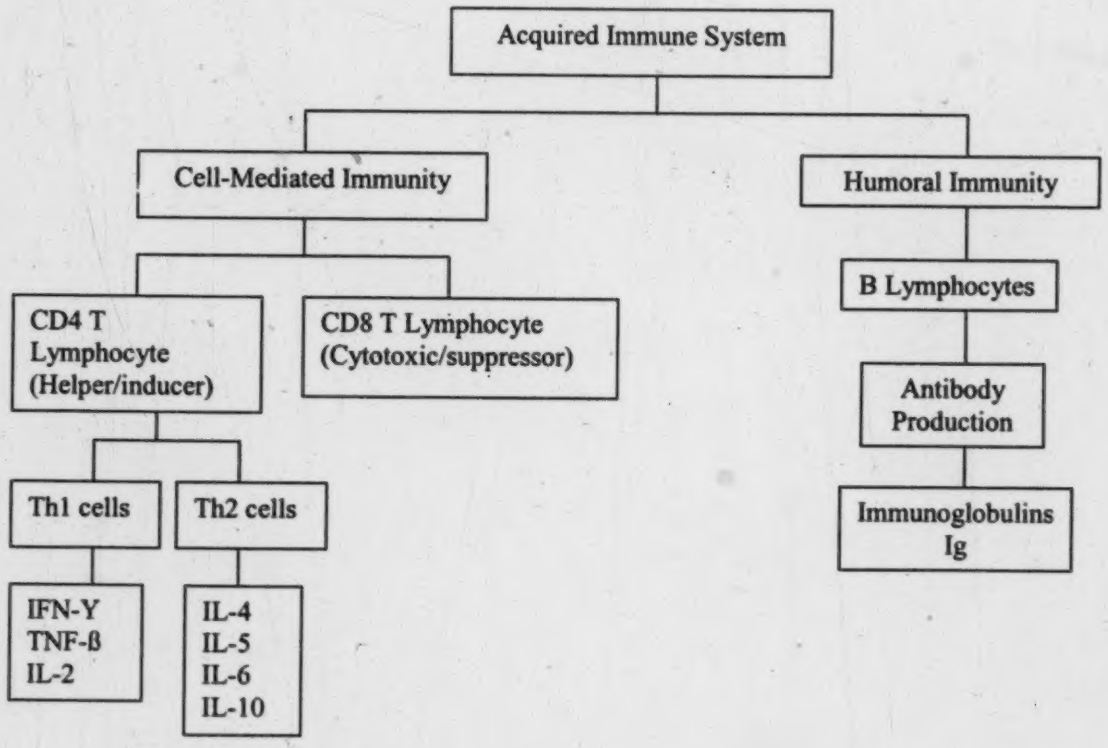
Acquired Immune System
Cell-Mediated Immunity
Humoral Immunity
CD4 T Lymphocyte (Helper/inducer)
CD8 T Lymphocyte (Cytotoxic/suppressor)
B Lymphocytes
Antibody Production
Th1 cells
Th2 cells
Immunoglobulins Ig
IFN-Y
TNF-ß
IL-2
IL-4
IL-5
IL-6
IL-10

Figure II - 2. Acquired Immune System

Adapted from Goldsby, R., T. Kindt, and B. Osborne. *Kuby Immunology*. New York: W.H. Freeman, 2000 and Leffell, M., A. Donnenberg, and N. Rose. *Handbook of Human Immunology*. New York: CRC Press, 1997, 640.

Soluble mediators of innate immunity include complement, acute phase proteins, and cytokines, which are further divided into interferons (IFN), interleukins (IL), colony stimulating factors (CSF) and tumor necrosis factors (TNF). IFNs are produced early in infection and are a first line of resistance to limit the spread of viral infections.[130] ILs are involved in directing other cells to divide and differentiate, CSFs are involved in direction division and differentiation of bone marrow-stem cells.[130] Finally, TNFs are important for mediating inflammation and cytotoxic reactions.[130] Complement proteins mediate phagocytosis, control inflammation, and interact with antibodies in the immune defense.[130] Physiochemical barriers include the skin and mucous membranes. Finally, the reticuloendothelial system functions to alleviate blood-borne infections through the activity of phagocytic cells.[55]

The cellular components of the innate immune system include phagocytes and natural killer (NK) cells. Phagocytes such as monocytes, macrophages, and granulocytes (neutrophils, basophils, and eosinophils) bind, engulf, and eliminate microbes and invading pathogens.[130] Macrophages are a first line of defence against microbes and malignancies due to their phagocytic, cytotoxic and intracellular killing capacities.[163] Neutrophils are the most abundance cellular component of the human immune system, constituting 60% of the circulating leucocytes,[123] with a total number of approximately 5 x 10^{11} cells in an individual weighing 70 kg.[123] Neutrophils are one of the first cells to arrive at sites of injury and infection.[123] Upon activation, neutrophils generate a range of toxic reactive oxygen species (ROS) and release proteolytic enzymes, which interact to kill infectious agents.[162] The neutrophil is also known to be involved with the synthesis and release of cytokines that influence both T-cell and B-cell activities.[85] In this way, the neutrophil plays an integral role in both the efferent (phagocytosis and degranulation) and afferent (release of immunomodulatory molecules) pathways of the immune response.[125] Natural killer cells lyse both tumor cell lines and those cells infected by viruses in absence of major histocompatibility complex (MHC) I and II antigen expression.[11] Resting NK cells express the ß chain of the IL-2 receptor, thus, direct stimulation of IL-2 results in activation of NK cells.[130] In addition, cytokines, INF-γ and TNF activate NK cells to increase their ability to lyse target cells.[11]

Acquired immune responses are classified into humoral immunity and cell-mediated immunity. Both are mediated by responses of distinct types of lymphocytes. In humoral immunity, B lymphocytes respond to foreign antigens by producing antibodies known as immunoglobulins (Ig). These immunoglobulins are soluble proteins that circulate in the blood and lymphatic system during an infection recognizing particular antigens, which they will bind to, deactivate, and destroy.[55] B-cells produce Ig for each specific antigen during an infection.

In cell-mediated immunity, T lymphocytes induce and promote intracellular destruction of microbes or lysis of infected cells.[11] T lymphocytes are further divided into CD4+ helper T cells and CD8+ cytotoxic T cells.[82] T lymphocytes do not produce antibody molecules, but instead, have an unusual specificity for antigens. They recognize peptide antigens attached to proteins encoded in the MHC expressed on surfaces of accessory cells.[11] In response to antigenic stimulation, CD4+ helper T cells secrete cytokines. Two distinct subsets of CD4+ helper T cells, Th1 and Th2, are defined by mutually exclusive cytokine secretion patterns.[82] Th1 produce IL-2, IFN-Y, and TNF-ß, while Th2 produce IL-4, IL-5, IL-6, and IL-10. These protein hormones promote proliferation and differentiation of T cells, B cells as well as macrophages.[11] Further, cytokines recruit and activate inflammatory leukocytes, providing an integral link between T lymphocyte immunity and innate immunity.[11] Cytotoxic CD8+ cells lyse cells that produce foreign antigens.

The mucosal-associated lymphatic system (MALT) is a network of immune structures at mucosal surfaces that provide protection at mucosal sites distal from the original site of antigen presentation.[27] The network is comprised of the gut-associated lymphoid tissue (GALT), urogenital tracts, lacrimal glands, lactating mammary glands, and in the respiratory tract, the bronchus-associated lymphoid tissue (BALT), salivary glands, and nasal-associated lymphoid tissue (NALT).[53] Mucosal immunity in connection with the innate non-specific defence forms the first line of defence against pathogens, allergens and antigens presented at mucosal surfaces.[53] Secretory IgA (sIgA) antibodies play a major role in effective specific immunity, as secretory IgA is the predominant immunoglobulin in mucosal secretion. SIgA has a parabolic relationship with age. At birth, levels of sIgA are undetectable, however there is a consistent increase with age. By 7 years, the levels of sIgA reach their approximate peak. The IgA antibodies are usually in secretory form consisting of dimeric IgA molecules joined by J chain and containing epithelial-derived protein, secretory component.[53] The distribution of the IgA subclasses varies at the different mucosal sites with IgA_2 as the predominant subclass in the distal gastrointenstinal tract (60%), while IgA_1 is predominately in the salivary glands (60-80%) and NALT (>90%).[53] Secretory antibodies play an integral role in defence against pathogenic microorganisms causing respiratory illness through immune exclusion at mucosal surfaces, intra-epithelial viral naturalization and immune elimination across mucosal surfaces.[27, 93] Individuals with reduced SIgA levels have been shown to have a higher incidence of infections.[71] A lower concentration of SIgA in saliva has been identified as a risk factor for upper respiratory tract infection (URTI) in children.[18]

Cortisol is the primary glucocorticoid secreted by the adrenal cortex. It presents a marked circadian rhythm; its average value drops 4 to 6 folds from the beginning of the day.[79] Its physiological effects are anti-inflammatory activity, blood pressure maintenance, and synthesis of carbohydrate from protein. Salivary cortisol has been confirmed to be a valid, reliable, and noninvasive indicator of the biological active free fraction of serum cortisol levels.[41] It has been shown that cortisol levels must be within normal physiological ranges for antibody production to occur during the inductive phase of the secondary response.[13] High levels of cortisol have been shown to inhibit antibody production in vitro.[13] In humans, cortisol appears to influence functional capabilities as well as the kinetics of various subpopulations of immunoregulatory cells, thereby affecting B cell function.[62] The immunosuppressive effects of cortisol have been shown to occur systemically, [45] but it is unclear whether higher than "normal" levels of cortisol directly affect the secretory immune system.[98]

II – 2. EXERCISE TRAINING AND THE IMMUNE SYSTEM

Over the past decade, a number of studies have demonstrated that moderate exercise appears to reduce risk of cardiovascular disease, promote health and fitness, decrease susceptibility to infection, and stimulate the function of various cell types in the immune system.[101, 144, 166] There is now substantial evidence that single bouts of exercise or acute effects and prolonged training over several weeks or chronic effects can produce relatively small but significant changes in the distribution and function of cellular and humoral components of the immune system. The theory in the field of exercise immunology is the "Inverted J Hypothesis" shown in **Figure II - 3.**[163] This theory suggests that an optimum dose of exercise results in decreased infectious disease incidence, reduced cancer incidence, and the enhancement of immune function.[163] On the contrary, exhaustive exercise or overtraining may lead to a compromised immune status, increased susceptibility to infection, and elevated risk of disease.[163, 166] In general, acute exercise bouts of moderate duration (<60 min) and intensity (<60% VO_2max) are associated with fewer perturbations and may have a lower impact on the normal functioning of the immune system than are prolonged, high intensity-sessions.[107]

Research in physical exercise in the healthy adolescent population has been shown to improve self-esteem,[24] decrease feelings of depression and anxiety,[108] improve physiological functioning,[17] and improve several immune parameters.[22, 47, 106, 118, 138]

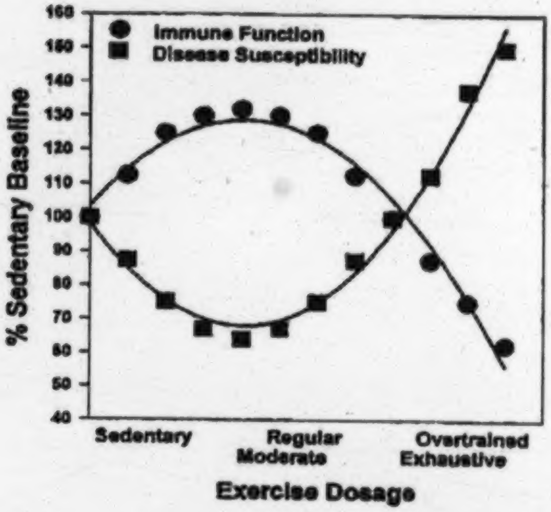

Figure II – 3. The Inverted J Hypothesis[163] Reprinted with permission from Woods JA, Davis JM, Smith JA, Nieman DC. Exercise and Cellular Innate Immune Function. Med Sci Sports Exerc. 1999;31(1):58.

II – 2a. Acute Exercise and Neutrophils

Numerous studies have revealed that acute exercise alters the concentration of neutrophils in blood.[113-115, 145, 163, 166] Many studies have reported an immediate increase in circulating neutrophils following exercise.[47, 106, 118, 137] Nemet and colleagues examined ten female participants aged 14-16 years following a water polo practise of 1.5 hours duration.[106] The acute exercise resulted in significant increases in granulocytes in blood.[106] Eliakim et al. reported enhanced cellular and humoral functions among both 10-12 year old highly trained female gymnasts and untrained girls with report of no differences between the two groups.[47] An acute bout of 20 minutes of treadmill running at a heart rate of 170-180 beats per minute caused an increase in neutrophils in blood.[47] Similarly, Perez and colleagues compared children aged 9-15 years on a cycle ergometer for 10x2 minute periods at constant intensity with children aged 9-11 years playing soccer to simulate real-life activity (approximately 40-50 minutes of vigorous aerobic exercise).[118] Both exercise types led to significant increases in granulocytes and all lymphocyte subpopulations.[118] Lastly, Shore and Shephard examined immune responses in 9 boys and 2 girls aged 10.3 ± 0.6 years subsequent to 30-minute acute bout of aerobic activity at 70-85% of the child's measured maximal heart rate per week.[137] Significant increases in circulating granulocyte and lymphocyte counts were observed.[137]

The effects of acute exercise on neutrophil function and the phagocytic process of the neutrophil have been reported in a limited number of studies. Using a variety of different measures and exercise protocols, some studies have shown an increase in neutrophil oxidative activity in response to exercise,[46, 68, 129, 141, 142] while others have reported decreases.[46, 49, 78, 84, 124] Despite the inconsistent findings, most researchers have concluded that moderate-intensity exercise, 60% of maximum oxygen uptake, elicits an enhanced functional activity of neutrophils and more intensive exercise results in suppressed neutrophil activity.[123, 125, 140]

There is an increase in the number of circulating neutrophils with exercise, likely due to demargination of cells from endothelial tissues, mediated by catecholamines.[123] The delayed rise several hours after the cessation of exercise is likely to be the result of a cortisol-induced release of mature neutrophils from the bone marrow into circulation or as part of the phagocytic and inflammatory response to exercise-induced tissue damage.[32] McCarthy and Dale (1988) proposed a model, which suggest that increases in plasma catecholamine levels induced by exercise act rapidly to demarginate and increase the proportion of neutrophils in circulation while the increases in plasma cortisol act after a 1 to 2 hour delay to release newly differentiated neutrophils from the bone marrow.[95] Subsequent work by McCarthy et al. (1992) supported this model with findings that post-exercise increases in plasma cortisol correlated with the magnitude of the delayed neutrophilia.[96]

Acute physical exercise has shown to increase circulating neutrophils in blood as well as neutrophil oxidative activity in healthy children and adolescents, which may be of clinical importance especially during periods of low immunity for the child with ALL during the maintenance phase due to chemotherapy treatments.

II – 2b. Acute Exercise and Salivary IgA

Regular exposure to exercise at a moderate level is believed to lead to a decrease in one's susceptibility to and incidence of contracting upper respiratory tract infection.[163] On the contrary, intense, prolonged exercise such as marathon running may increase the incidence of upper respiratory tract infection.[98] Tomasi et al have suggested that exercise-induced decreases in sIgA, the first line of defence against pathogenic viruses, may contribute to the increased incidence of URTI.[149] Further, Mackinnon et al found that episodes of URTI were preceded by 22-27% decreases in sIgA concentrations following exercise, and suggested that large decreases in mucosal IgA during exercise may be related to this increase incidence of infections in elite athletes.[88] Studies of elite or high performance athletes generally indicate that intense endurance exercise results in lower levels of sIgA sampled immediately after exercise.[31, 51, 105, 149] A recent study of elite women rowers has reported that salivary IgA concentration decreased by 50% after 2 hours of rowing training.[105] Similarly, 40-60% decreases in sIgA concentration were observed after 2 to 3 hours of competitive cross-country skiing,[149] and marathon running.[103] There is evidence of a cumulative effect of repeated high-intensity prolonged training undertaken by elite athletes on mucosal immunity.[53] Clinical experience with athletes suggests that repeated high-intensity training can cause mucosal immune suppression, which is characterized by a slower rate of recovery of sIgA to baseline with successive days of training.[53]

Several studies have also investigated the effects of moderate intensity exercise in recreational athletes, moderately trained individuals rather than highly trained elite athletes, using the treadmill or cycle ergometer in a laboratory setting. This investigation is significant given the broad spectrum of the exercising public. Several studies have reported that brief, moderate intensity exercise fails to elicit any significant post-exercise change in salivary IgA concentration.[21, 28, 87, 97] This may be of clinical importance for the child undergoing maintenance treatment for acute lymphoblastic leukemia as patients are slowly returning to normal activities such as riding bicycles, playing street hockey or a soccer game with peers. It is unknown if physical exercise will decrease muscosal IgA in a child with ALL undergoing maintenance therapy. If brief, moderate intensity exercise does not elicit a significant decrease in sIgA, risk of upper respiratory tract infection in these patients may be reduced.

II – 2c. Acute Exercise and Salivary Cortisol

Increasingly, salivary cortisol is being used as a valid, non-invasive alternative to the assessment of cortisol in blood. Several studies have shown that physical effort such as exercise can increase plasmatic or salivary cortisol. However, few studies have been performed in children. In a study conducted by del Corral et al., ten healthy male children performed an acute submaximal exercise bout for 30 minutes at 70% VO_{2max}, determined by a graded exercise test.[41] Blood and saliva samples were obtained at 15 minutes and 30 minutes of steady-state exercise, as well as 15 minutes post-exercise. Exercise significantly increased serum cortisol levels at 15 minutes and 30 minutes, as well as 15 minutes post-exercise compared with resting values.[41] Salivary cortisol response increased by as much as 81% above resting level, however, did not achieve statistical significance.[41] Nonetheless, it is important to note that significant correlations were observed between saliva and serum measurements, ranging from r =.77 to r = .90 during and after exercise.[41] Further, Lac et al., analyzed salivary cortisol during 30-minute submaximal exercise on 9 students aged 19-23 years.[79] The load was fitted so that heart rate remained at 170 ± 4 beats per minute, corresponding to approximately 70% VO_{2max}.[79] Cortisol was sampled every 5 minutes over the acute bout and then at 10 minutes post-exercise, 30 minutes post-exercise, 1 hour 30 minutes, and 5 hours post-exercise.[79]

Results indicated a significant increase from the first step of exercise and remained steady until the end of the test.[79] A delayed increase appeared following cessation of exercise followed by a decrease at 1 hour 30 minutes.[79] Values did not return to reference values until 5 hours post-exercise.[79] In another study conducted by O'Connor and Corrigan, 8 males with a mean age of 22.9 ± 2.3 years exercised on a bicycle ergometer for 30 minutes at an intensity equal to 75% of their VO_{2max}.[109] Saliva and blood samples were obtained at 15 minutes pre-exercise, immediately prior to the onset of exercise, during exercise at 15 minutes, post-exercise, and 15 minutes post-exercise.[109] A significant increase was found in both serum and salivary cortisol above the resting control values post-exercise and 15 minutes post-exercise.[109] The response of salivary cortisol to an acute bout of exercise was found to be similar to the serum cortisol response in both pattern and timing.[109]

II – 3. PEDIATRIC CANCER AND EXERCISE

A limited amount of research has been conducted in childhood cancer patients with respect to physical activity and/or exercise. However, a study conducted by Martinson and Liu reported three wishes of a child with cancer. Of the sample, 40% of pre-school aged children indicated that they wanted to return to play activities following 4.3 months of cancer treatment, however 83% wished they could play following 26 months.[92] From the school-aged children, 25% indicated they wanted to play and 38% wished to exercise after 4.3 months.[92] Follow-up at 26 months demonstrated that 20% wanted to play and 20% wanted to exercise.[92] Further, Keats et al examined the relationship between leisure time physical activity (LTPA) and psychosocial well-being amongst 53 adolescent cancer survivors.[77] LTPA data revealed four main activity patterns across the cancer experience: maintainers (active at all time points), temporary relapsers (active prediagnosis, inactive during treatment, active posttreatment), permanent relapsers (active prediagnosis, inactive during treatment, inactive posttreatment), and nonparticipants (inactive at all three time periods).[77] Analysis revealed maintainers of physical activity gave superior scores on pyschosocial well-being such as depression, physical abilities, general self, physical appearance, opposite sex relations, same sex relations.[77] Thus, physical activity patterns during the adolescent cancer experience may be related to psychosocial well-being. Wright et al described self-perceptions of physical activity in 62 children and adolescent survivors of acute lymphoblastic leukemia with 71 comparable healthy participants.[164] The survivors had significantly poorer self-perceptions of their adequacy in and prediction for physical activity than the healthy young people, however, they were similar in their enjoyment of physical exercise.[164] It was suggested that survivors of ALL are at risk for avoiding physical activity and are thus less likely to reap potential physical and psychological benefits. Lastly, Robertson & Johnson suggest prescribing exercise for children after cancer to facilitate recovery, performance and development with consequent improvement in quality of life and long-term outcome.[128] Shore & Shephard examined 12 weeks of aerobic training in six children with cancer.[138] This is the only study to date examining immune responses to exercise in children treated for cancer. All six participants underwent initial and final exercise tests, however, only three participants completed the 12-week training program comprising of three 30-minute aerobic exercise sessions at 70-85% of the child's maximum heart rate.[138] The 12-weeks of exercise training resulted in a decrease in leukocytes, lymphocytes and granulocytes, similar to that seen in normal children, however the counts were much lower in the children who were receiving chemotherapy.[138] Initial resting data in these children already showed depression in many of the immune parameters including total leukocyte count and lymphocytes.[138]

II – 4. SUMMARY

Research has shown that cancer and cancer treatment cause adverse physiological, psychological, functional, and immunomodulatory effects in children. Parents may believe physical activity to be harmful to the child's immune system during treatment for acute lymphoblastic leukemia. As a result, parents may impose limitations on their child's daily activities including schooling and physical exercise. However, it is during the maintenance phase of treatment for acute lymphoblastic leukemia that children are returning to the classroom or playing with fellow peers and trying to regain the normalcy of childhood. Thus, the overprotective nature of parents may in fact lead to further psychological problems for the young cancer patient. Physical exercise may be one intervention to enhance overall well-being and immune status. Previous studies have reported the potential benefits of quality of life in children diagnosed with cancer. However, it is unknown whether or not physical exercise is beneficial or detrimental to the child's immune system, causing increased susceptibility to infection during cancer treatment. Oncologists do not encourage or discourage exercise in children with ALL, as the role of exercise in the recovery of the immune system during maintenance cancer therapy is not well understood. This study is the first study to examine this issue in ALL survivors on maintenance therapy.

III: CHAPTER THREE

METHODS AND PROCEDURES

III – 1. ETHICAL CONSIDERATIONS

The present study proposal was presented to and approved by the Health Research Ethics Board (HREB) Biomedical Panel (A) Committee in the Faculty of Medicine and Dentistry at the University of Alberta **(Appendix B)** as well as by the Northern Alberta Clinical Trials and Research Centre for the Northern Alberta Pediatric Program (NAPP) **(Appendix C)**. Information about the study was outlined in a participant package, which included a letter of information, a blood draw consent form, a consent form and/or an assent form if participant was under the age of 18 years, which outlined the right to withdraw, confidentiality, and the risks and benefits involved in the study **(Appendix D)**. Participants were asked to sign all of the above forms. Non-participation in this study did not affect accessibility to assessment and treatment at the Outpatient Oncology Clinic of the Stollery Children's Hospital (SCH). The risk to the participant in participating in this study was minimal and all precautions were taken in order to ensure safety of the participant. The risk to participation was a small chance of skin reaction (allergy) to the electrodes during the maximal aerobic graded exercise test and/or tape, band-aids applied during or after the blood sampling procedure. Participants were free to withdraw from the study at any time without prejudice or coercion.

III – 2. PARTICIPANTS

A convenience sample of children and adolescents aged 7 – 18 years with acute lymphoblastic leukemia receiving maintenance treatment was used. Potential participants were identified by a Clinical Research Nurse in the Northern Alberta Pediatric Oncology Program at the Stollery Children's Hospital. The SCH provides assessment and treatment for all childhood cancer patients in Northern Alberta.

III – 3. SAMPLE SIZE

From the literature in physical exercise and immune function and cancer, sample sizes range from 6 to 70 participants. It was approximated that 10 children at the SCH fit the eligibility criteria and were on maintenance treatment for ALL, of which, it was hoped that all 10 patients would be recruited into the study. These participants would be age- and gender-matched with 10 healthy controls. Based upon the time commitment of two visits and the requirement of blood sampling via butterfly catheter needle, a recruitment rate of 75% was anticipated.

III – 4. INCLUSION CRITERIA

The participants who participated in this study were aged 7 – 18 years diagnosed with acute lymphoblastic leukemia. Participants were receiving treatment during the maintenance phase. For the purposes of the study, participants were required to be able to undergo exercise testing as determined by the pediatric oncologist. In addition, participants were required to be willing to travel to the exercise facility for testing on two separate occasions. Consent and approval to participate was obtained from the referring pediatric oncologist and participants as well as parents/guardians provided signed Informed Consent and/or Assent.

III – 5. STUDY DESIGN

This study was a nonrandomized controlled trial. This experimental design was chosen to include a comparison group for the ALL patients and match them according to age and gender to allow for comparison in some relevant ways (i.e. maturity level, physical fitness). Both groups experienced the same conditions and environments, thus, the most likely reason for group differences or change from pre to post testing is due to the physical exercise and the existing group difference of acute lymphoblastic leukemia and receiving cancer therapy versus healthy participants. Effects such as maturation were expected to be equal in both groups.

All participants performed two exercise visits and were scheduled for testing at the same time of the day. Two exercise physiologists conducted VO_{2peak} testing using the same protocol **(Appendix E)** and the same metabolics measurement system. All VO_{2peak} testing occurred at the Cross Cancer Institute (CCI) and all submaximal exercise with blood and saliva sampling occurred at the Behavioural Medicine Fitness Centre with the Project Director conducting all exercise testing sessions. The blood sampling was provided primarily by one clinical nurse. One other nurse assisted, as needed, in the insertion of the butterfly catheter. The blood and salivary assays were conducted by the Project Director who was trained in the measurement and methodology protocols. The 24-hour nutritional recall was performed by two students in the Faculty of Agriculture, Nutrition and Forestry at the University of Alberta under the supervision of a registered dietician. Both students were trained on how to interact with the young participants and used the same environment, methodology and approach to assess food intake from the prior day.

III – 6. PROCEDURE

Upon approval from the pediatric oncologist, the Project Director presented a recruitment package to each patient and parent or guardian during clinic at the Stollery Children's Hospital. The package included a detailed cover letter outlining the objectives of the study, a questionnaire, two consent forms, two child assent forms, and two blood draw consent forms **(Appendix D)**. The Project Director discussed the study in further detail and was available to answer any questions the parent and/or patient had regarding participation in the study. The participant and parent were asked to approach a friend to participate in the study to act as an age- and gender-matched control. This was optional. If the parent and child could name a friend to approach, a second recruitment package was given to present to the parent of the friend. This package contained the same questionnaire and forms, however, included a different cover letter **(Appendix D)**. The parent or guardian of the friend was asked to contact the project director if interested in the study.

If the parent or child did not feel comfortable or could not name a friend to participate, a child of the same age and gender was recruited through word of mouth in the Faculty of Physical Education. Staff who were parents of eligible children were provided with information regarding the study and asked to discuss participation in the study with their child. Those interested were then asked to contact the Project Director for a friend recruitment package and to schedule exercise testing sessions.

The project director followed-up with patients via telephone call within one week of the clinic visit. Verbal confirmation of a patient's interest in the study was obtained over the telephone from the parent or participant. After that, participants were scheduled for two visits, a graded maximal exercise test on visit 1 and a submaximal exercise test with blood and saliva collection on visit 2. Participants and/or parents were required to sign and bring one copy of each of the informed consent, child assent, blood draw consent, and questionnaire package to testing on visit 1.

III – 6a. Graded Maximal Aerobic Exercise Test

Exercise testing was performed at the Cross Cancer Institute in Edmonton, Alberta. Anthropometric data including height and weight was recorded on visit 1 of testing at the CCI. Body weight and standing height was determined without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared. An exercise trainer conducted all measurements. A physician supervised 12 lead electrocardiogram (ECG) graded maximal aerobic exercise test was performed to exhaustion or a symptom-limited peak was achieved on a treadmill (Quinton ClubTrack) using the Modified Balke protocol[70] **(Appendix E)**. The participants were not permitted to hold the handlebar of the treadmill during the test and were encouraged to reach a level of maximal exertion. ECG was not be utilized for healthy control participants. The test was terminated when the participant refused to continue despite verbal encouragement or if the ECG detected any cardiovascular abnormalities.

Expired gases were analyzed continuously for oxygen and carbon dioxide concentration by a metabolic measurement system (Medgraphics). Volume and gas calibration were performed before each test. Heart rate was monitored throughout the test (Polar Beat Heart Rate Monitor, Polar USA Inc., Stanford, Connecticut) and was recorded at rest, at the end of each minute of exercise, at maximal exercise and at 2 minutes recovery. Blood pressure was taken pre-exercise and immediately post-exercise and recorded on the Exercise Testing Form **(Appendix F)**.

The following criteria for maximal exercise were those used in progressive incremental cardiopulmonary exercise testing in children[12, 75] and adults[66]: 1) exhaustion of the participant or inability to continue walking on the treadmill despite verbal encouragement, 2) predicted maximum heart rate (HRmax) achieved [210 – (0.65 x age) $\pm$ 10%], we considered the predicted HRmax to have been achieved if the HR recorded was $\geq$ 90% of the predicted value, and 3) maximal respiratory exchange ratio (RERmax) of > 1.0. Two of these criteria must have been satisfied for maximal exercise and peak VO_2 to be achieved.

III – 6b. Acute Exercise Bout

Submaximal exercise testing on visit 2 was performed at the Behavioural Medicine Fitness Centre in Edmonton, Alberta. In order to control for circadian variations in the circulating concentration of all cells and cortisol, all submaximal exercise testing was scheduled in the morning at 9:00 a.m. in the fasting state. All of the participants were instructed to perform a 10-hour fast prior to testing on visit 2 and not to exercise for at least 48 hours prior to the blood collection and exercise test. Participants were also given EMLA cream to be placed on the inside of the elbow 1-hour prior to their exercise appointment. The EMLA cream was given to numb the area of skin so that the participant did not feel the needle being inserted. If the ALL participant had a friend participate in the study, both were scheduled for their exercise session plus blood and saliva sampling on the same day. Participants performed an acute bout of exercise consisting of an intermittent jog/walk on a treadmill (Quinton ClubTrack) for 30 minutes.

Participants performed a jog for the first 10 minutes of the session followed by 10 minutes of walking and finally 10 minutes of jogging to reach a total exercise duration of 30 minutes. The first 5 minutes on the treadmill was a warm-up period whereby the work rate was gradually increased to the participant's target heart rate. The jogging portion was performed at anaerobic threshold (AT) determined by the V-slope method.[161] When VCO_2 (L/min) is plotted against VO_2 (L/min), the intercept or the break-point where the two slopes coincide is the AT measured by gas exchange.[161] This break-point was determined by placing a 45 right triangle on the plot.[161] The walking portion was performed at an intensity of 70% of the VO_{2peak} measured on visit 1 from the graded aerobic maximal exercise test. This moderate intensity level and intermittent exercise was chosen as it compared to regular activities of children in the age range of 7-18 years (i.e. a hockey or soccer game). Furthermore, this level of intensity is believed to be appropriate in order to elicit a favourable immune response, more specifically, enhanced neutrophil functional capacity. Levels of intensity during the exercise bout were monitored via heart rate (Polar Beat Heart Rate Monitor, Polar USA Inc., Stanford, Connecticut) and recorded every 5 minutes during the acute exercise bout. Participants performed a five-minute cool down following the acute bout. Exercise intensities and heart rates were recorded using the Submaximal Exercise Testing Form **(Appendix G)**.

III – 6c. Blood Collection

On the second visit, prior to the exercise session, participants met at the Outpatient Oncology unit at the Stollery Children's Hospital to have a butterfly catheter inserted into the antecubital vein for the purpose of obtaining repeated blood samples. A qualified nurse inserted the butterfly needle while the participant was seated. The butterfly remained in the arm for the duration of the entire session, approximately four hours. A fasted blood sample was drawn first thing in the morning. Following which, participants were given a standardized breakfast comprised of a protein bar and orange juice. This breakfast was calculated based on the number of calories required for one meal according to the recommended intake of energy based on the participant's body weight and age.[10] The calculation was determined as follows: total kcal per day divided by 3 meals per day, subtract 250 kcal for morning snack = kcal requirement for standard breakfast. Further blood samples were drawn pre-exercise, immediately post-exercise, 1 hour post-exercise and 2 hours post-exercise in a seated position. Five mL of blood was drawn at each time from each participant in one 5-mL tube containing anticoagulant ethylenediamine tetraacetic acid (EDTA). Samples were chilled on ice during blood collection and were analyzed within two hours of sampling.

III – 6d. Saliva Collection

On visit 2 of testing, saliva sampling was performed immediately following the collection of the blood sample at the same time points: fasting, before exercise bout, immediately after, 1 hour post-intervention and 2 hours post-intervention. The procedure to collect saliva required the participants to chew on a piece of sterile cotton Salivettes (Sarstedt) for a 1-minute period, which was chilled on ice during collection and then frozen at –20°C until assayed. To prevent dilution of the samples, fluid intake was restricted 10 minutes prior to saliva collection.

III – 7. DATA COLLECTION

III – 7a. Variables

The dependent variables in this study were assessed in peripheral blood and saliva: neutrophil count and function, salivary IgA and salivary cortisol.

The independent variable in this study was exercise. Samples were repeated in each participant over 5 time points including fasting, pre-exercise, post-exercise, 1-hour post exercise and 2-hours post exercise. Measurements were taken at these time points to examine the effect of the acute exercise bout over time and to determine if there was any difference in response between the two groups.

Intervening variables that may account for variations in immune variables are diet and normal diurnal fluctuations. To determine the potential effect of a change in nutritional intake between groups, a dietetic intern assessed each of the participant's diet in a 24-hour nutrition recall. All participants were also provided with a standardized breakfast based on their daily required intake for one meal according to their age, height and weight. In addition, all measurements were made at approximately the same time of day, each time, for all participants.

III – 7b. Demographic Information

Age, grade at school, maturation level via Tanner stage and past exercise history via Godin Leisure-Time Exercise Questionnaire were noted via Questionnaire **(Appendix D)**. Date of birth, date of diagnosis, type of treatment protocol and venous access device were recorded from medical charts using the Participant Tracking Sheet **(Appendix H)**.

III – 7c. Measurement

III – 7c – i. CBC and Neutrophil Assay

Whole blood (3 mL) treated with EDTA was analyzed for total white blood cells, neutrophils, monocytes, lymphocytes, hemoglobin and hematocrit with a Beckman Coulter A^{c}T 5 diff Autoanalyzer (Mississauga, ON) to determine complete blood counts (CBC).

Neutrophil function was examined using dihydrorhodamine 123 (DHR) as a fluorescent indicator in flow cytommetric analysis of oxidative burst.[156] Blood samples were centrifuged at 3000rpm for 10 minutes. Two vials of plasma per participant were aliquoted and stored at –70°C for future analysis. Four mL of lysis buffer was added to the RBC pellet and was incubated for 10-15 minutes at 37°C. Sample was thoroughly mixed and centrifuged at 1500 rpm for 5 minutes. Supernatant was discarded in bleach waste and an additional 4 mL of lysis buffer was added to the pellet and the above steps repeated. Supernatant was discarded and 2 mL of wash buffer was added. Sample was thoroughly mixed and centrifuged at 1500 rpm for 5 minutes. Supernatant was discarded and 800 uL of wash buffer was added. 500 uL of cell suspension was removed and 1.8ul DHR was added. The sample was incubated for 5 minutes in a water bath. 100ul was removed from the sample and placed on ice. 100ul of phorbol myristate acetate (PMA) was added and again incubated for an additional 15 minutes in a waterbath. 100ul of the sample was removed at 5 minutes and 10 minutes and placed on ice. The samples were covered for protection from light and were taken to a lab for analysis. The flow cytometer used was a FacScan Becton Dickinson (Sunnyvale, CA). The NeutAcq template was used with the Sue Neut Set for correct settings. The sample was acquired within two hours of completing the assay. 20,000 gated neutrophils were analyzed. Figure III – 1 and Figure III – 2 below show the flow cytometry print outs at pre-exercise before any PMA stimulation and at stimulation Time 15.

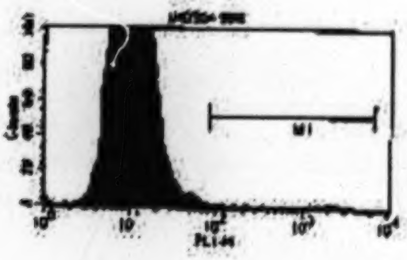

Figure III – 1. No Stimulation

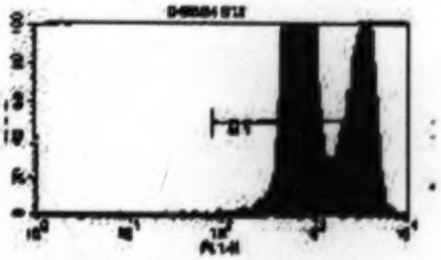

Figure III – 2. Stimulation Time 15

III – 7c – ii. Salivary IgA Assay

Salivary secretory IgA was assayed using an indirect enzyme immunoassay (Salimetrics, LLC). Samples were thawed completely and centrifuged at 1500 x g (@ 3000 rpm) for 15 minutes. Clear samples were pipetted into appropriate tubes. 30 uL of 1x sIgA diluent was pipetted in tubes 2 through 6 labelled. The standard was diluted by adding 15 uL of the 600 ug/mL standard tube 1 to tube 2. The sample was mixed well. Changing pipetted tips, 15 uL was removed from tube 2 to 3 and mixed well. This was continued for remaining tubes 4-6. Final concentrations of standards for tubes 1 through 6 were 600 ug/mL, 200 ug/mL, 66.7 ug/mL, 22.2 ug/mL, 7.4 ug/mL, and 2.5 ug/mL. 3 mL of 1 x sIgA diluent was pipetted into a tube and set aside for later. One small tube was labelled with the identity of each sample. With a repeater pipette, 100 uL of 1x sIgA diluent was added into each tube. 25 uL of saliva was pipetted into the appropriate tube. One 12 x 75 mm snap-cap tube was labelled for each calibrator, control, sample and one tube for the zero value. Using a repeator pipette, 4mL of 1x sIgA diluent was added into each tube. 10 uL of calibrator, control or diluted saliva sample was added to the appropriate tube. 10 uL of saliva was added to 40 uL of diluent. The antibody-enzyme conjugate was diluted 120x by adding 25 uL of the conjugate to the 3 mL of 1x sIgA diluent prepared earlier. This was mixed well and 50 uL of the diluted antibody-enzyme conjugate was pipetted to all tubes using a repeator pipette. Each tube was gently vortexed and incubated for 90 minutes at room temperature. Each tube was again gently vortexed and 50 uL of solution from above was added to the microtire plate according to the template. 50 uL of 1x sIgA diluent was added to the non-specific binding (NSB) wells. Plate was covered with the adhesive plate sealer and was incubated at room temperature with continual mixing at 500 rpm for 90 minutes. The plate was washed 6 times with 1x wash buffer. Washing was done by gently adding wash buffer into each well by pipetting 300 uL of wash buffer into each well and then decanting the liquid into a sink. After each wash, the plate was thoroughly blotted on paper towels before turning upright. 50 uL of tetramethylbenzidine (TMB) solution was added to each well with a multichannel pipette. The plate was mixed on a plate rotator for 5 minutes at 500 rpm and incubated in the dark at room temperature for an additional 40 minutes. 50 uL of stop solution was added with a multichannel pipette. The plate was then mixed on a plate rotator for 3 minutes at 500 rpm until no green colour remained. The bottom of the plate was wiped dry with a water-moistened lint-free cloth and read in a plate reader at 450 nm within 10 minutes of adding stop solution (correction at 492 to 620 is desirable). Concentrations were multiplied by 5 to obtain final sIgA concentrations in ug/mL. The coefficient of variance for this assay was 20.3%.

III – 7c – iii. Salivary Cortisol Assay

Saliva samples were assayed for salivary cortisol by a high sensitivity enzyme immunoassay kit (Salimetrics, LLC). Samples were thawed completely and centrifuged at 1500 x g (@ 3000 rpm) for 15 minutes. The plate layout was determined. 24 mLs of assay diluent was pipetted into a disposable tube and was set aside for later step. 25 uL of standards and unknowns was pipetted into appropriate wells. Standards and samples were assayed in duplicate. 25 uL of assay diluent was pipetted into 2 wells to serve as the zero. 25 uL of assay diluent was pipetted into each NSB well. A 1:1,600 dilution on the conjugate was made by adding 15 uL of the conjugate to the 24 mL of assay diluent prepared earlier. The diluted conjugate solution was immediately mixed. 200 uL was pipetted into each well using a multichannel pipette. The plate was mixed on a plate rotator for 5 minutes at 500 rpm and incubated at room temperature for an additional 55 minutes. The plate was then washed 4 times with 1X wash butter. 300 uL of wash buffer was pipetted into each well. Liquid was discarded by inverting plate over sink. After each wash, the plate was blotted thoroughly on paper towels before turned upright. 200 uL of TMB solution was added to each well with a multichannel pipette. The plate was mixed on a rotator for 5 minutes at 500 rpm and incubated in the dark at room temperature for an additional 25 minutes. 50 uL of stop solution was added with a multichannel pipette and the plate was mixed again on a rotator for 3 minutes at 500 rpm. The bottom of the plate was wiped off with a water-moistened lint-free cloth and read in a plate reader at 450 nm within 10 minutes of adding stop solution (correction at 492 to 620 is desirable). The coefficient of variance for this assay was 13.0%.

III – 7d. Assumptions

The demographics and classification of the patients who agreed to participate in this study were assumed to not differ from those who did not agree to participate. Reasons for refusal of participation included time constraints, transportation or distance, and a lack of interest in the study. As general population characteristics are not known, the results of this study are only generalizable to a population with similar characteristics.

III – 8. STATISTICAL ANALYSIS

III – 8a. Participant Demographic Information

Descriptive statistics were used to describe the control group ($n = 6$) and ALL group ($n = 4$) on basic demographics and inferential statistics (t-test independent samples) were used to determine comparability of the groups. An alpha level of 0.100 was used for the basic demographics as it represents reasonable protection against committing a Type I error. Significant differences were reported for all ps < 0.100 due to the exploratory, pilot nature of the study and being underpowered due to a small sample size.

III – 8b. Results of Acute Exercise Bout

Descriptive statistics were used to describe the results of the treatment within each group and inferential statistics were used to determine if differences occurred between the groups. An alpha level of 0.100 was used for analyzing results due to a small sample size and underpowered study. Salivary cortisol and IgA were analyzed for normal distribution, kurtosis and skewness. A log transformation was performed if the data were not normally distributed for statistical analysis. Repeated measures analysis of variance (ANOVA) and t-test independent samples were the methods of analysis used to compare the effects of the acute exercise bout on neutrophil function measures (i.e. neutrophil size, granularity and oxidative burst) complete blood count measures, absolute neutrophil count and salivary measures, IgA and cortisol over time and between the groups. Dependent t-test analyses were performed on the complete blood count differentials found significant over time to determine where significant change occurred. Bivariate correlations were performed between salivary cortisol and sIgA, salivary cortisol and neutrophil percentage and salivary cortisol and absolute neutrophil count to determine any correlations between the hormonal and immune variables.

IV: CHAPTER FOUR

RESULTS

IV – 1. FLOW OF PARTICIPANTS THROUGH THE STUDY

Participants were recruited from February 2004 to August 2004. Figure IV – 1 presents the flow diagram of participants through each stage of the study. A total of 10 participants with ALL receiving maintenance treatment were deemed eligible to participate in the study. Two participants elected not to participate in the study. Two participants lived out of town and were not due for a clinic visit during the recruitment period and thus, were mailed a package and instructed to contact the Project Director if interested in the study. The primary reasons for non-participation were cited as transportation and distance and/or non-interest in the study. Of the remaining eligible participants, six agreed to participate and were enrolled in the study, however, one participant relapsed prior to testing and required new treatment, thus becoming ineligible and one participant withdrew from the study prior to the maximal aerobic graded exercise test. Therefore, four participants with ALL underwent testing. All participants were male and had the Pre-B ALL subtype.

Two ALL participants approached a friend to participate in the study as age- and gender-matched controls. However, one of the control participants could not provide blood sampling due to the experience of a needle for the first time and thus, salivary samples were acquired only. As a result, another age- and gender-matched healthy participant was recruited. The research nurse had difficulty finding one participant's vein. This participant was rescheduled for visit 2 testing, however, was no longer on maintenance treatment at the time of the exercise test. In another instance, two participants, one ALL participant and one healthy control performed testing on the same day, however, the FacScan flow cytometer had technical difficulties and failed to perform the analysis on the blood samples. Only one participant, the ALL participant, could be rescheduled to come in for testing and blood sampling again. As a result, the matched healthy control participant provided saliva samples, CBC and ANC measures, however, no neutrophil function measures could be attained. Thus, another healthy control participant was recruited.

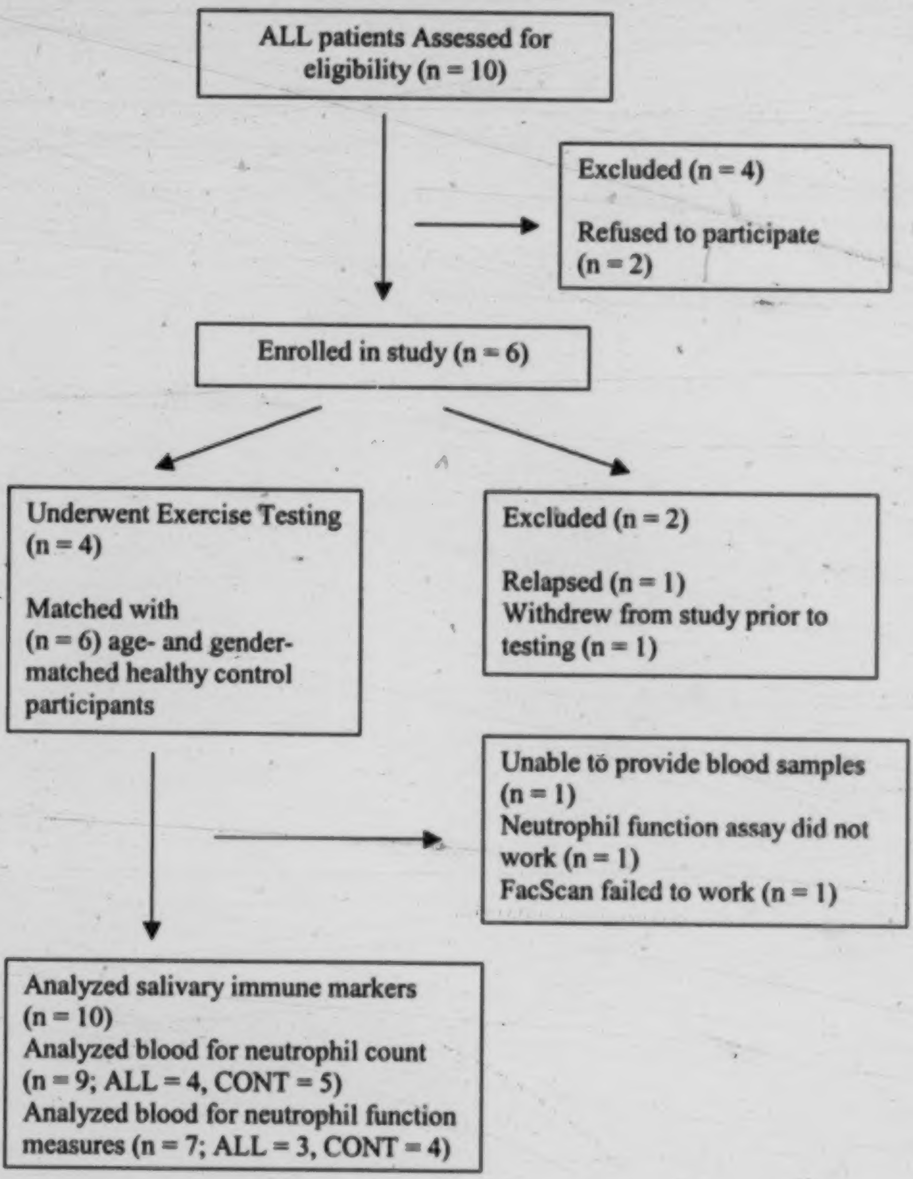
ALL patients Assessed for eligibility (n = 10)
Excluded (n = 4)
Refused to participate (n = 2)
Enrolled in study (n = 6)
Underwent Exercise Testing (n = 4)
Matched with (n = 6) age- and gender-matched healthy control participants
Excluded (n = 2)
Relapsed (n = 1)
Withdrew from study prior to testing (n = 1)
Unable to provide blood samples (n = 1)
Neutrophil function assay did not work (n = 1)
FacScan failed to work (n = 1)
Analyzed salivary immune markers (n = 10)
Analyzed blood for neutrophil count (n = 9; ALL = 4, CONT = 5)
Analyzed blood for neutrophil function measures (n = 7; ALL = 3, CONT = 4)

Figure IV – 1. Flow diagram of participants through the study

IV – 2. PARTICIPANT CHARACTERISTICS

IV – 2a. Demographic Information

Table IV – 1 presents the demographic and regular exercise information for participants completing the study ($n = 10$). No significant differences between the groups ($p < 0.100$) were found. Both groups were similar in age, school grade, height and tanner stage. However, a 19.1 kg difference in weight was found between the groups, the ALL group being heavier than the control group. Further, the body mass index (BMI) was found to be 4.6 kg/m^2 higher in the ALL group than the control group. Both weight and BMI were not found to be significant, likely due to the small sample size and low power of the study. Of particular note, were the differences in the total exercise minutes and in the breakdown of strenuous, moderate and mild minutes reported between the ALL group and the control group. The control participants reported 275 more minutes of total physical activity per week and consistently reported more exercise minutes at all three intensity levels. The greatest differences were found in the strenuous and mild minutes reported, the controls participating in 259 more minutes of strenuous activity and 99 more minutes of mild activity than the ALL group.

Table IV – 1. Participant Characteristics

Characteristic	ALL (n = 4)	CONT (n = 6)	p
Age (years)	11.3 (5.3)	10.8 (4.6)	0.898
School grade	5.5 (5.4)	5.5 (4.7)	1.0
Height (cm)	148.8 (31.9)	141.3 (24.3)	0.686
Weight (kg)	62.0 (44.9)	42.9 (21.8)	0.473
BMI (kg/m^2)	24.2 (9.7)	19.9 (4.1)	0.449
Tanner stage	2.0 (2.4)	1.7 (2.1)	0.822
Weekly exercise (mins)			
Total exercise	215.4 (174.0)	490.8 (401.8)	0.239
Strenuous exercise	32.5 (10.9)	291.0 (372.9)	0.289
Moderate exercise	152.5 (128.2)	208.0 (123.2)	0.530
Mild exercise	51.3 (59.5)	150.0 (55.7)	0.104

Data are presented as the mean ± (standard deviation)

IV – 2b. Fitness Data

The fitness data for all participants from the graded maximal aerobic exercise test on visit 1 is shown in Table IV – 2 and the maximal fitness data for both the groups is shown in Table IV – 3. Peak exercise results revealed that only one ALL participant achieved a maximal heart rate according to his age predicted maximum and two ALL participants achieved maximal respiratory exchange ratios greater than 1.0. On the contrary, four out of six control participants achieved their age predicted maximal heart rate and all control participants achieved RERs > 1.0. Significant differences were found in peak duration of the graded maximal aerobic test and the relative VO_{2peak} in ml/kg/min. The control group walked for 3.3 minutes longer on the graded maximal aerobic test and achieved a VO_{2peak} of 12.5 ml/kg/min higher than the ALL group. However, according to the VO_{2peak} criteria defined in Chapter III – 6a, two participants in the ALL group did not attain VO_{2peak} whereas all participants in the control group did attain VO_{2peak}. Absolute VO_{2peak} is theoretically different between the groups, however, due to the small sample size of the study, it did not show significance. Similarly, the control participants reached higher maximal heart rates and maximal respiratory exchange ratios than the ALL participants, however, again, no significance was found.

Table IV – 2. Maximal Fitness Data for all Participants

Group Age	Predicted HRmax (bpm)	Predicted HR range (bpm)	HRmax Achieved (bpm)	RERmax Achieved	VO_{2peak} (ml/kg/min)	VO_{2peak} (L/min)	Peak Achieved?
ALL 7	205 +/- 20.5	185 - 226	164	0.93	31.9	0.71	No
ALL 7	205 +/- 20.5	185 - 226	162	1.02	36.0	0.88	Yes
ALL 13	201 +/- 20.1	181 - 221	175	0.99	25.9	2.31	No
ALL 18	198 +/- 19.8	178 - 218	181	1.17	25.1	2.78	Yes
CONT 7	205 +/- 20.5	185 - 226	167	1.06	38.4	0.93	Yes
CONT 7	205 +/- 20.5	185 - 226	190	1.08	47.8	1.08	Yes
CONT 7	205 +/- 20.5	185 - 226	165	1.03	31.7	0.74	Yes
CONT 13	201 +/- 20.1	181 - 221	184	1.03	42.3	2.35	Yes
CONT 13	201 +/- 20.1	181 - 221	200	1.27	49.8	3.42	Yes
CONT 18	198 +/- 19.8	178 - 218	207	1.42	43.3	2.72	Yes

Table IV – 3. Maximal Fitness Data

Fitness Variable	ALL (n = 4)	CONT (n = 6)	p
Peak duration (mins)	6.9 (1.8)	10.2 (2.5)	0.054
Relative VO_{2peak} (ml/kg/min)	29.7 (5.2)	42.2 (6.6)	0.013
Absolute VO_{2peak} (L/min)	1.67 (1.03)	1.87 (1.11)	0.780
Heart Rate max (beats/min)	170.5 (9.0)	185.5 (17.1)	0.150
Respiratory Exchange Ratio max	1.03 (.10)	1.15 (.16)	0.223

Data are presented as the mean ± (standard deviation)

IV – 2c. Nutrition Recall

The results of the 24-hour nutrition recall are shown in Table IV – 4. The nutritional analysis showed small but significant differences in the mean percent kcal from protein and dietary fibre between groups. The healthy control participants consumed significantly (3%) more calories from protein and significantly more grams from dietary fibre (6.7g) in their diet. None of the other nutrients differed between groups.

Table IV – 4. Nutrition Record

Variable	ALL (n = 4)	CONT (n = 6)	p
Total Calories (kcal)	1981 (361)	2327 (733)	0.412
Calories from Fat (kcal)	589 (84)	727 (449)	0.491
Calories Saturated Fat (kcal)	230 (24)	230 (24)	0.677
Protein (g)	76.2 (58.5)	81.2 (36.4)	0.870
% kcal from Protein	11 (2)	14 (2)	0.035
Carbohydrate (g)	257.7 (35.9)	323.7 (80.3)	0.167
% kcal from Carbohydrate	53 (12)	53 (12)	0.640
Fat (g)	65.4 (9.4)	81.0 (50.3)	0.489
% kcal from Fat	31 (6)	30 (11)	0.917
Dietary Fibre (g)	11.0 (3.7)	17.7 (6.4)	0.099
Total Sugars (g)	118.7 (51.2)	134.4 (36.5)	0.583

Data are presented as the mean ± (standard deviation)

IV – 2d. Fasting Measures

Fasting measures were analyzed separately and are shown in Table IV – 5. Significant differences between the ALL and control group were found in the relative percent of neutrophils and lymphocytes, absolute lymphocyte count and eosinophil concentration in blood ($p < 0.05$). The ALL participants had a higher relative percentage of neutrophils, whereas the healthy control participants had a higher relative percent of lymphocytes and greater lymphocyte and eosinophil concentration at fasting.

Table IV – 5. Fasting Measures

Variable	ALL (n = 4)	CONT (n = 5)	p
White Blood Cells (x 10^9L)	4.2 (1.5)	4.9 (1.4)	0.445
Red Blood Cells (x 10^{12}L)	4.1 (.6)	4.6 (.6)	0.235
Hemoglobin (g/L)	131 (19.7)	132 (13.6)	0.962
Hematocrit (L/L)	.38 (.06)	.39 (.04)	0.753
Neutrophil Concentration (%)	62.7 (13.0)	36.0 (7.1)	0.006
Absolute Neutrophil Count (/L)	2.73 (1.33)	1.86 (.79)	0.256
Lymphocyte Concentration (%)	22.9 (11.1)	46.2 (6.7)	0.006
Absolute Lymphocyte Count (/L)	.84 (.25)	2.21 (.47)	0.001
Monocyte concentration (%)	9.27 (1.3)	8.48 (1.18)	0.365
Eosinophil concentration (%)	3.95 (1.03)	7.44 (1.21)	0.003
Basolphil concentration (%)	1.18 (.42)	1.86 (.64)	0.110
Salivary IgA (ug/mL)	66.2 (47.0)	77.8 (90.3)	0.843
Salivary cortisol (ug/dL)	.79 (.08)	.82 (.09)	0.664

Data are presented as the mean ± (standard deviation)

IV – 3. RESULTS OF ACUTE EXERCISE

IV – 3a. Complete Blood Count

Results of the complete blood count from pre-exercise to 2-hours post exercise are shown in Table IV – 6. A significant main effect for Time ($p < 0.100$) was found in white blood cells, red blood cells, hemoglobin (HGB), hematocrit (HCT), relative neutrophil (Neut%) and lymphocyte (LY%) percent, absolute neutrophil and lymphocyte count (ALC) and eosinophil concentration (Eos%). The ANC and ALC were calculated by multiplying the respective relative percentages by the WBC count, derived from the CBC, for each participant at each time point. A dependent paired samples t-test analysis was performed on the above differentials to determine at which sampling time points changes occurred. These results are shown in Table IV – 7. A significant increase in WBCs ($p = 0.002$), RBCs ($p = 0.058$), HGB ($p = 0.074$) HCT ($p = 0.060$), ANC ($p = 0.006$) and ALC ($p = 0.003$) and significant decrease in eosinophil concentration ($p = 0.003$) occurred from pre to post exercise in both groups. A significant difference was found during the recovery period, whereby a decrease was observed from post-exercise to 1-hour in the WBCs, RBCs, HGB, HCT, ANC, ALC as well as the relative lymphocyte percent ($p < 0.100$). A significant decrease was also observed in WBCs, RBCs, HGB, HCT, ALC, relative lymphocyte percent, and eosinophil concentration from post-exercise to 2-hours post exercise ($p < 0.100$). A significant increase in relative neutrophil percent was found from post-exercise to 1-hour post and 2-hours post exercise ($p < 0.05$). Lastly, a significant increase was observed in ANC from 1-hour to 2-hours post exercise ($p = 0.036$). Refer to the graph in Figure IV – 2 for the changes in neutrophil concentration for both groups over time. Although changes in hematocrit were statistically significant, they were not great enough to influence blood fluid cell measurements.

A significant main effect for Group was observed in relative neutrophil percent ($p = 0.019$), relative lymphocyte percent ($p = 0.018$), ALC ($p = 0.000$) and eosinophil concentration ($p = 0.003$). On average, the relative neutrophil percent of the ALL group was 24% greater while the relative lymphocyte percent was 20% lower than the control group over the four sampling time points. This is consistent with what was observed at fasting levels for both variables. The percent of neutrophils in the control group increased by approximately 2% following the acute bout of exercise. A further increase of 5% and 6% was observed at 1-hour and 2-hours recovery from pre-exercise levels. Conversely, the relative percent in the ALL participants decreased from pre to post-exercise by approximately 2% followed by a 4% increase at 1-hour post exercise and a further 3% increase by 2-hours post exercise from pre-exercise values. However, the relative neutrophil percent of the control group was not within the normal range at pre and post-exercise and the relative lymphocyte percent was not within the normal range for the ALL group from post to 2-hours post exercise. Lastly, the control group showed a 38% greater eosinophil concentration and a 60% greater lymphocyte concentration compared to the ALL group over the four sampling time points. There were no significant Time by Group interactions ($p > 0.100$).

Table IV – 6. Complete Blood Count

Variable	Pre	Post	1-hour	2-hour	Normal Range	Group F	Group p	Time F	Time p	Time X Group F	Time X Group p
WBC (x 10^9/L)					4.0 –11.0 x 10^9/L	.7	.434	8.1	.023	.7	.606
ALL	4.7 (2.0)	6.1 (2.3)	4.5 (1.4)	5.0 (1.6)							
CONT	5.0 (1.5)	6.8 (1.7)	5.7 (1.4)	6.2 (1.8)							
RBC (x 10^{12}/L)					4.5-6.5 x 10^{12}/L	2.5	.155	6.4	.037	1.1	.405
ALL	4.0 (.5)	4.3 (.7)	4.0 (.6)	4.0 (.6)							
CONT	4.7 (.5)	4.8 (.5)	4.6 (.4)	4.6 (.4)							
HGB (g/L)					130-180 g/L	.1	.803	9.1	.018	1.1	.418
ALL	130 (15)	138 (23)	128 (20)	132 (19)							
CONT	136 (10)	138 (11)	133 (10)	132 (9)							
HCT (L/L)					.400-.540 L/L	.2	.680	5.5	.049	1.2	.396
ALL	.38 (.05)	.41 (.07)	.38 (.06)	.39 (.06)							
CONT	.41 (.03)	.41 (.04)	.40 (.03)	.40 (.03)							
Neutrophil (%)					45-70%	9.3	.019	3.7	.097	2.8	.14
ALL	65.6 (14.2)	64.1 (14.4)	69.5 (10.0)	68.6 (8.4)							
CONT	39.9 (8.2)	42.0 (12.0)	45.3 (13.4)	46.3 (13.5)							
Absolute Neutrophil Count (10^9/L)											
ALL	3.29 (1.99)	4.15 (2.22)	3.20 (1.27)	3.50 (1.36)	2.0-7.5 x 10^9/L	.8	.402	4.0	.084	.5	.694
CONT	2.05 (.90)	3.02 (1.29)	2.73 (1.23)	3.06 (1.48)							

Variable	Pre	Post	1-hour	2-hour	Normal Range	Group F	Group p	Time F	Time p	Time X Group F	Time X Group p
Lymphocyte (%)					20.0-40.0%	9.4	.018	4.2	.078	.9	.519
ALL	21.1 (11.5)	22.9 (12.1)	19.1 (7.8)	19.3 (6.1)							
CONT	43.3 (7.2)	42.1 (11.2)	39.0 (12.5)	39.1 (11.7)							
Absolute Lymphocyte Count (10^9/L)					1.5-4.0 x 10^9/L	75.8	.000	13.4	.008	.6	.669
ALL	.82 (.15)	1.20 (.37)	.78 (.18)	.89 (.20)							
CONT	2.09 (.48)	2.74 (.42)	2.09 (.31)	2.28 (.14)							
Monocyte (%)					3.0-10.0%	.2	.685	1.2	.399	1.5	.325
ALL	7.0 (2.1)	7.7 (1.6)	7.4 (1.9)	8.0 (1.4)							
CONT	7.7 (1.5)	8.1 (1.3)	8.1 (1.1)	7.6 (1.5)							
Eosinophil (%)					1.0-5.0%	19.0	.003	16.1	.005	1.7	.287
ALL	5.2 (.6)	4.1 (1.2)	3.0 (.8)	3.2 (1.8)							
CONT	7.7 (.8)	5.9 (.6)	6.0 (.8)	5.3 (1.2)							
Basophil (%)					0.0-0.5%	2.0	.199	1.1	.417	.8	.565
ALL	1.2 (.6)	1.3 (1.0)	.9 (.4)	1.0 (.5)							
CONT	1.4 (.3)	1.9 (.8)	1.5 (.4)	1.6 (.7)							

Data are presented as the mean ± (standard deviation); ALL = 4; CONT = 5

Table IV – 7. Paired Samples T-Test for Complete Blood Count

Pair	p
WBC Pre - WBC Post	0.002
WBC Pre - WBC 1-Hour	0.486
WBC Pre - WBC 2-Hours	0.217
WBC Post - WBC 1-Hour	0.002
WBC Post - WBC 2-Hours	0.055
WBC 1-Hour - WBC 2-Hours	0.067
RBC Pre - RBC Post	0.058
RBC Pre - RBC 1-Hour	0.137
RBC Pre - RBC 2-Hours	0.583
RBC Post - RBC 1-Hour	0.001
RBC Post - RBC 2-Hours	0.010
RBC 1-Hour - RBC 2-Hours	0.266
HGB Pre - HGB Post	0.074
HGB Pre - HGB 1-Hour	0.182
HGB Pre - HGB 2-Hours	0.705
HGB Post - HGB 1-Hour	0.001
HGB Post - HGB 2-Hours	0.011
HGB 1-Hour - HGB 2-Hours	0.376
HCT Pre - HCT Post	0.060
HCT Pre - HCT 1-Hour	0.205
HCT Pre - HCT 2-Hours	0.800
HCT Post - HCT 1-Hour	0.003
HCT Post - HCT 2-Hours	0.018
HCT 1-Hour - HCT 2-Hours	0.253
Neut % Pre - Neut % Post	0.787
Neut % Pre - Neut % 1-Hour	0.070
Neut % Pre - Neut % 2-Hours	0.059
Neut % Post - Neut % 1-Hour	0.010
Neut % Post - Neut % 2-Hours	0.032
Neut % 1-Hour - Neut % 2-Hours	0.888
ANC Pre - ANC Post	0.006
ANC Pre - ANC 1-Hour	0.375
ANC Pre - ANC 2-Hours	0.185
ANC Post - ANC 1-Hour	0.058
ANC Post - ANC 2-Hours	0.412
ANC 1-Hour - ANC 2-Hours	0.036

Pair	p
LY % Pre - LY % Post	0.965
LY % Pre - LY % 1-Hour	0.158
LY % Pre - LY % 2-Hours	0.143
LY % Post - LY % 1-Hour	0.017
LY % Post - LY % 2-Hours	0.059
LY % 1-Hour - LY % 2-Hours	0.849
ALC Pre - ALC Post	0.003
ALC Pre - ALC 1-Hour	0.759
ALC Pre - ALC 2-Hours	0.371
ALC Post - ALC 1-Hour	0.000
ALC Post - ALC 2-Hours	0.015
ALC 1-Hour - ALC 2-Hours	0.160
Eos % Pre - Eos % Post	0.003
Eos % Pre - Eos % 1-Hour	0.000
Eos % Pre - Eos % 2-Hours	0.001
Eos % Post - Eos % 1-Hour	0.176
Eos % Post - Eos % 2-Hours	0.050
Eos % 1-Hour - Eos % 2-Hours	0.426

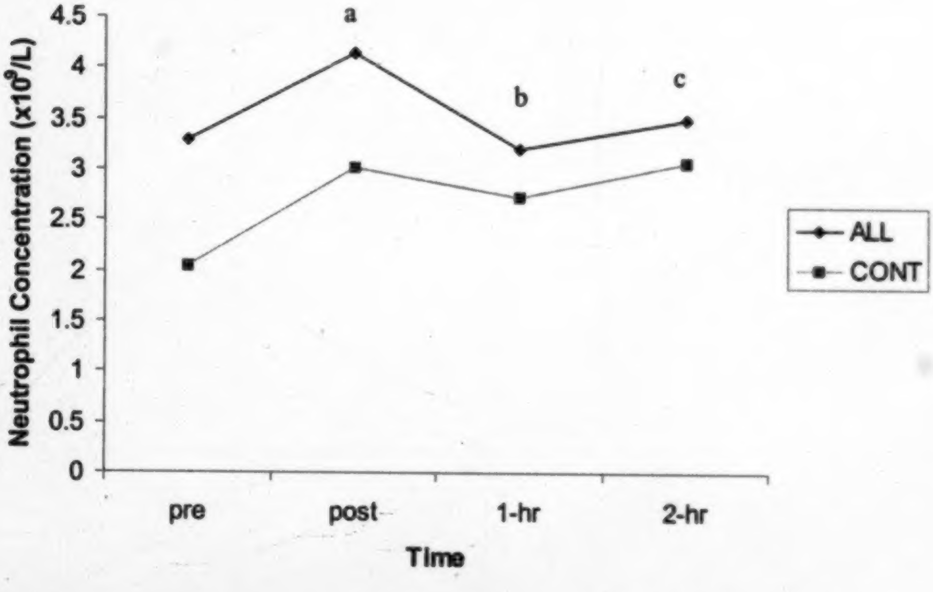

Figure IV – 2. Absolute Neutrophil Count. [a]Significantly different from pre ($p < 0.05$). [b]Significantly different from post ($p < 0.100$). [c]Significantly different from 1-hour ($p < 0.50$).

IV – 3b. Neutrophil Function Measures

Results of the acute bout of exercise on neutrophil function are presented in Table IV – 8. Size and granularity of neutrophils at Time 0 or the basal state was reported. No significant main effects were observed for Time, Group or Time by Group interaction ($p > 0.100$) in both variables. Neutrophil size was consistently greater at all four sampling time points in the ALL group as was granularity compared to the control group. Oxidative burst was reported at Time 0 with no PMA stimulation and at Time 5, Time 10 and Time 15 with PMA stimulation. A significant main effect for Group ($p = 0.029$) was found only at Time 0 with the control participants having significantly more neutrophils producing reactive oxygen species than the ALL participants at all four sampling time points. Figures IV – 3, 4, 5 and 6 show the oxidative burst at all stimulation time points for pre-exercise, post-exercise, 1-hour post and 2-hours post exercise, respectively. At the subsequent stimulation time points, the ALL group responded to the PMA producing more free radicals than the control group. The oxidative burst of the control group levelled off at stimulation time 10 while the ALL group levelled off at stimulation time 15. The ratio of neutrophils producing reactive oxygen species at Time 5, 10 and 15 showed significant main effects for Group ($p = 0.048$, 0.074 and 0.050, respectively) as well as a significant main effect for Time by Group interaction at Time 15 ($p = 0.006$). Table IV – 9 shows a comparison of the ratios at all time points between the two groups. No significant differences were observed ($p > 0.100$) except for Ratio 5 at pre-exercise ($p = 0.084$).

Table IV – 8. Neutrophil Function Measures

Variable	Pre	Post	1-hour	2-hour	Group		Time		Time X Group	
					F	p	F	p	F	p
Size (time 0)					2.5	.175	2.6	.228	.3	.827
ALL	404.2 (134.8)	443.1 (130.4)	452.9 (133.5)	431.9 (119.2)						
CONT	324.6 (64.9)	313.7 (72.2)	337.3 (57.2)	328.7 (58.7)						
Granularity (time 0)					.6	.460	1.1	.467	.6	.659
ALL	760.9 (171.5)	813.4 (140.0)	814.0 (122.1)	818.8 (136.3)						
CONT	678.6 (210.7)	723.8 (227.7)	677.6 (219.6)	680.3 (203.6)						
Oxidative Burst (time 0)					9.2	.029	1.2	.435	2.7	.218
ALL	13.4 (7.59)	18.5 (5.11)	20.7 (2.68)	21.3 (4.90)						
CONT	44.9 (21.1)	51.6 (29.2)	39.0 (12.7)	46.2 (28.0)						
Oxidative Burst (time 5)					.5	.512	2.3	.259	1.1	.476
ALL	376.5 (320.3)	493.7 (374.5)	493.1 (259.8)	384.8 (416.3)						
CONT	285.7 (353.9)	312.4 (436.5)	291.5 (224.0)	268.1 (177.2)						
Oxidative Burst (time 10)					1.0	.375	4.9	.111	1.3	.410
ALL	854.2 (545.4)	911.8 (613.8)	989.1 (649.6)	750.3 (632.3)						
CONT	513.1 (678.3)	316.0 (384.2)	546.0 (508.4)	487.6 (572.8)						
Oxidative Burst (time 15)					2.0	.216	2.8	.208	.7	.621
ALL	854.7 (639.6)	1004.7 (535.9)	1085.6 (681.5)	970.4 (645.2)						
CONT	410.3 (544.2)	282.8 (269.3)	554.5 (401.6)	552.4 (492.4)						

Variable	Pre	Post	1-hour	2-hour	Group		Time		Time X Group	
					F	p	F	p	F	p
Ratio (time 5)					6.8	.048	.3	.812	1.7	.345
ALL	33.8 (25.7)	27.3 (18.8)	24.4 (13.1)	18.9 (20.1)						
CONT	6.0 (5.9)	4.4 (3.9)	7.4 (4.1)	7.2 (4.6)						
Ratio (time 10)					5.1	.074	2.7	.220	2.4	.242
ALL	94.4 (81.1)	55.3 (38.9)	49.8 (33.9)	35.2 (30.7)						
CONT	10.7 (11.0)	4.8 (3.4)	13.1 (8.1)	13.0 (11.6)						
Ratio (time 15)					6.6	.050	1.8	.313	41.2	.006
ALL	82.6 (59.1)	63.2 (47.6)	54.8 (36.9)	44.2 (29.4)						
CONT	8.5 (9.1)	4.7 (2.6)	13.8 (6.2)	14.6 (10.5)						

Data are presented as the mean ± (standard deviation); ALL n = 3; CONT n = 4

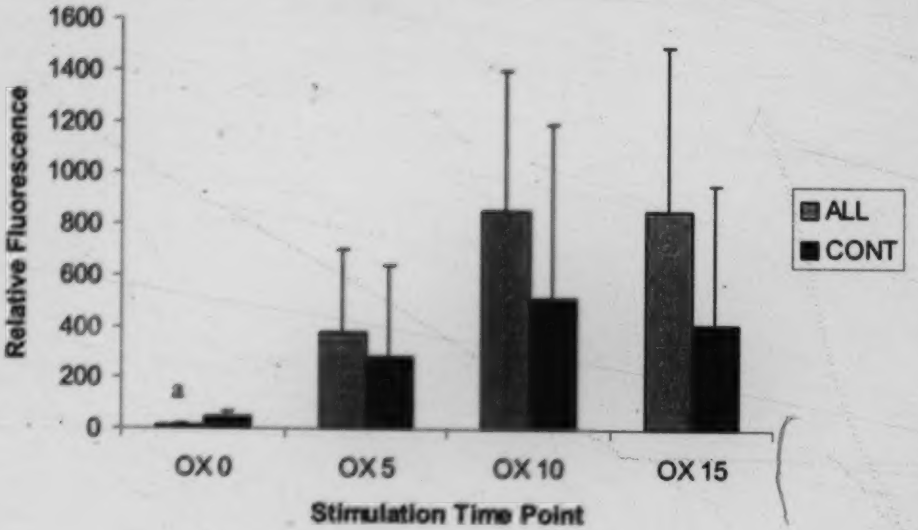

Figure IV – 3. Neutrophil Oxidative Burst Pre-Exercise. [a]Significantly different than CONT group ($p < 0.05$)

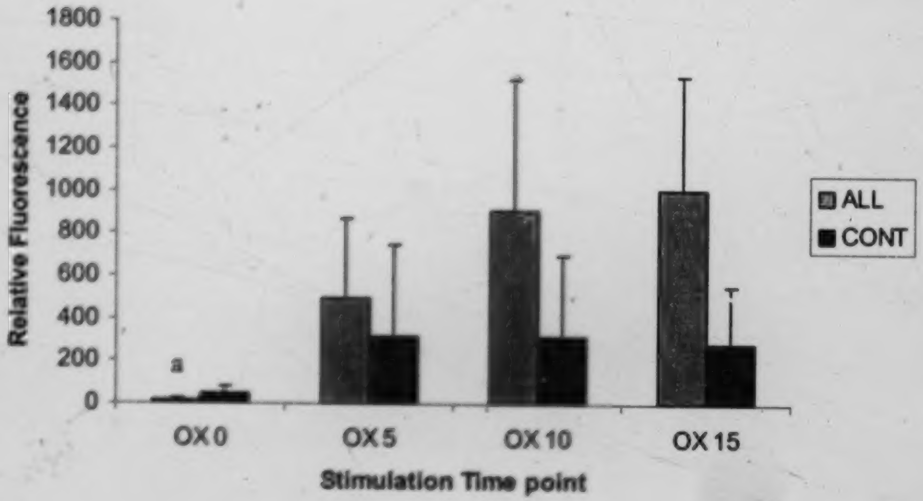

Figure IV – 4. Neutrophil Oxidative Burst Post Exercise. [a]Significantly different than CONT group ($p < 0.05$)

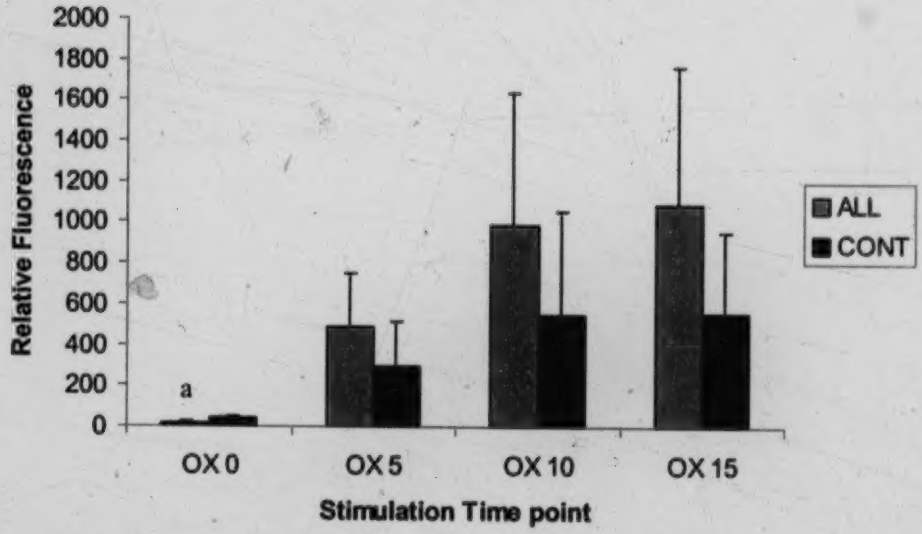

Figure IV – 5. Neutrophil Oxidative Burst 1-Hour Post Exercise. [a]Significantly different than CONT group ($p < 0.05$)

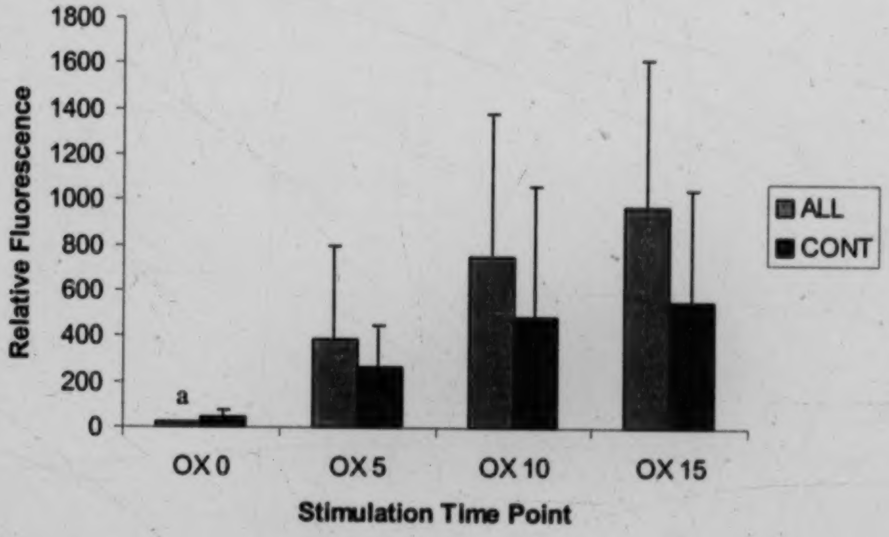

Figure IV – 6. Neutrophil Oxidative Burst 2-Hours Post Exercise. [a]Significantly different than CONT group ($p < 0.05$)

Table IV – 9. Neutrophil Ratio Measures

Ratio Time Point	ALL (n = 3)	CONT (n = 4)	p
Ratio (time 5)			
Pre	33.8 (25.7)	5.9 (5.9)	0.084
Post	27.3 (18.8)	4.4 (3.9)	0.166
1-hour	24.4 (13.1)	7.4 (4.1)	0.144
2-hours	18.9 (20.1)	7.2 (4.6)	0.419
Ratio (time 10)			
Pre	94.3 (81.1)	10.7 (11.0)	0.215
Post	55.3 (38.9)	4.8 (3.4)	0.152
1-hour	49.7 (33.9)	13.1 (8.1)	0.198
2-hours	35.2 (30.7)	13.0 (11.6)	0.336
Ratio (time 15)			
Pre	82.6 (59.1)	8.5 (9.1)	0.160
Post	63.2 (47.6)	4.7 (2.6)	0.167
1-hour	54.8 (36.9)	13.8 (6.2)	0.192
2-hours	44.2 (29.4)	14.6 (10.5)	0.115

Data are presented as the mean ± (standard deviation)

IV – 3c. Salivary Measures

IV – 3c – i. Salivary IgA

The salivary IgA results were not normally distributed, thus log transformations of the data were performed for statistical analysis. The results of the effects of the moderate intensity exercise on salivary IgA are presented in Table IV – 10. No significant main effects were found for Time or Group, and no Time by Group interactions was observed ($p > 0.100$). Figure IV – 7 presents the salivary IgA response across the four time points for all participants. The ALL group demonstrated a trend towards an increase immediately following exercise, which continued until 2-hours post exercise where it slightly decreased, however remained above pre-exercise levels. The control group exhibited a slight decrease at post-exercise from pre-exercise levels with a small increase at 1-hour and a considerable increase at 2-hours post exercise. The graph in Figure IV – 8 illustrates the sIgA responses of each participant. Participants responded similarly with the exception of two participants, one control aged 13 and one ALL participant aged 18 years. Most of the participants responded with a slight increase in sIgA levels following the acute bout of exercise, which then levelled off or remained stable by 2-hours post exercise.

IV – 3c – ii. Salivary Cortisol

The salivary cortisol results were normally distributed. The results did not show significant main effects for Time, Group or a Time by Group interaction ($p > 0.100$). Table IV – 10 presents these results. Figure IV – 9 shows the mean salivary cortisol response for both groups of participants and Figure IV – 10 demonstrates the salivary cortisol response in all participants over the four sampling time points. Salivary cortisol levels demonstrated a trend towards an increase immediately following acute exercise to 2-hours post exercise in the ALL group. Similarly, there was a trend towards an increase in cortisol levels in the control group from pre-exercise to post-exercise, which continued to 1-hour post exercise followed by a plateau at 2-hours post exercise. Table IV – 11 shows Pearson correlations performed to determine a correlation between the following three pairs of variables: salivary cortisol and sIgA, salivary cortisol and neutrophil concentration and salivary cortisol and relative neutrophil percent. No significant correlations were observed except for salivary cortisol and neutrophil concentration during recovery at post-exercise and 1-hour post exercise. An inverse relationship was found such that as cortisol levels increased, neutrophil concentration decreased at post-exercise and 1-hour post-exercise. This decrease in neutrophil concentration was demonstrated in the graph shown in Figure IV – 2.

Table IV – 10. Salivary Measures

Variable	Pre	Post	1-hour	2-hour	Group		Time		Time X Group	
					F	p	F	p	F	p
IgA (ug/mL)					.1	.134	1.3	.36	1.0	.906
ALL	53.1 (36.4)	88.2 (61.4)	158.0 (148.7)	142.2 (96.7)						
CONT	97.1 (130.8)	90.5 (54.0)	100.0 (87.3)	271.9 (523.9)						
Cortisol (ug/dL)					.0	.835	1.6	.277	.3	.849
ALL	.72 (.18)	.85 (.07)	.90 (.02)	.93 (.01)						
CONT	.74 (.33)	.87 (.09)	.92 (.03)	.92 (.03)						

Data are presented as the mean ± (standard deviation); ALL = 4, CONT = 6
Salivary IgA data were not normally distributed and log transformation was performed prior to performing statistical analysis.

Table IV – 11. Pearson Correlation of Salivary Cortisol with IgA and Neutrophils

	sIga Pre	sIgA Post	sIgA 1-Hour	sIgA 2-Hours	Neut % Pre	Neut % Post	Neut % 1-Hour	Neut % 2-Hours	ANC Pre	ANC Post	ANC 1-Hour	ANC 2-Hours
Cortisol Pre	-.092 p =.801				-.192 p =.621				-.188 p =.627			
Cortisol Post		.121, p =.739				-.524 p =.148				-.599 p =.088*		
Cortisol 1-Hour			-.483 p =.158				-.560 p =.117				-.599 p =.088*	
Cortisol 2-Hours				.511 p =.131				-.166 p =.669				-.243 p =.530

Salivary measures N = 10 (ALL = 4, CONT = 6); Neut % and ANC measures N = 9 (ALL = 4, CONT = 5)
* Correlation is significant at the 0.100 level (2-tailed)

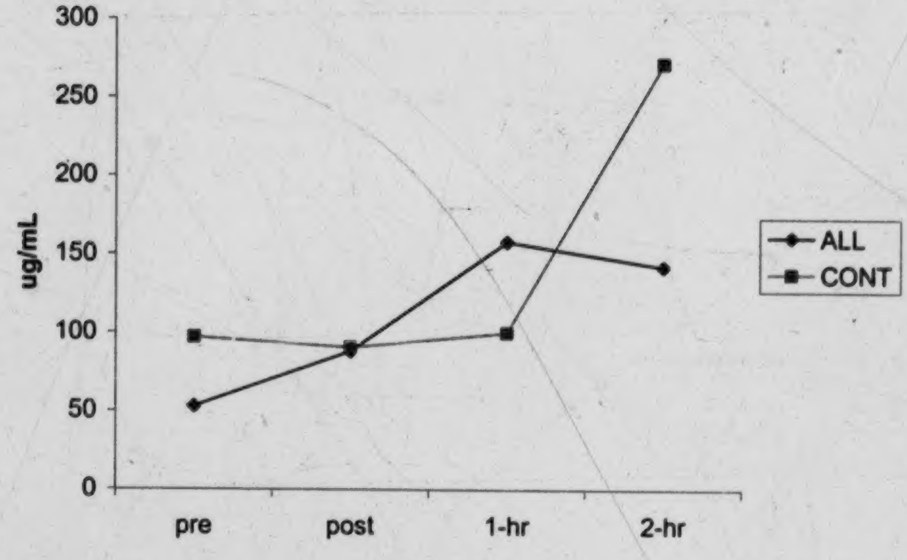

Figure IV – 7. Mean Salivary IgA Response

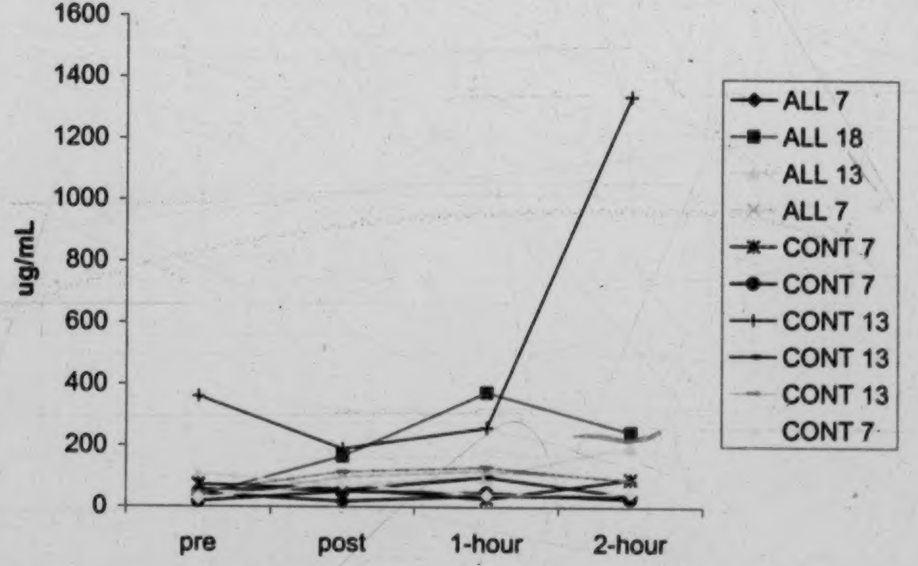

Figure IV – 8. Salivary IgA Response

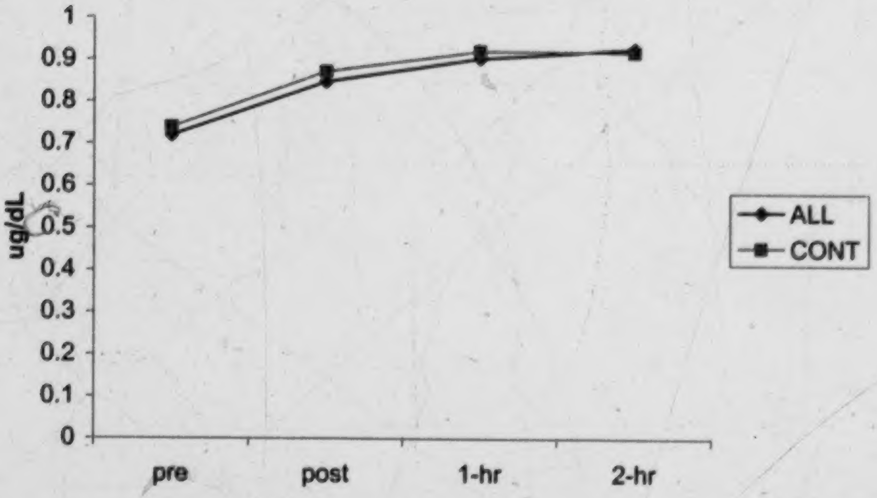

Figure IV – 9. Mean Salivary Cortisol Response

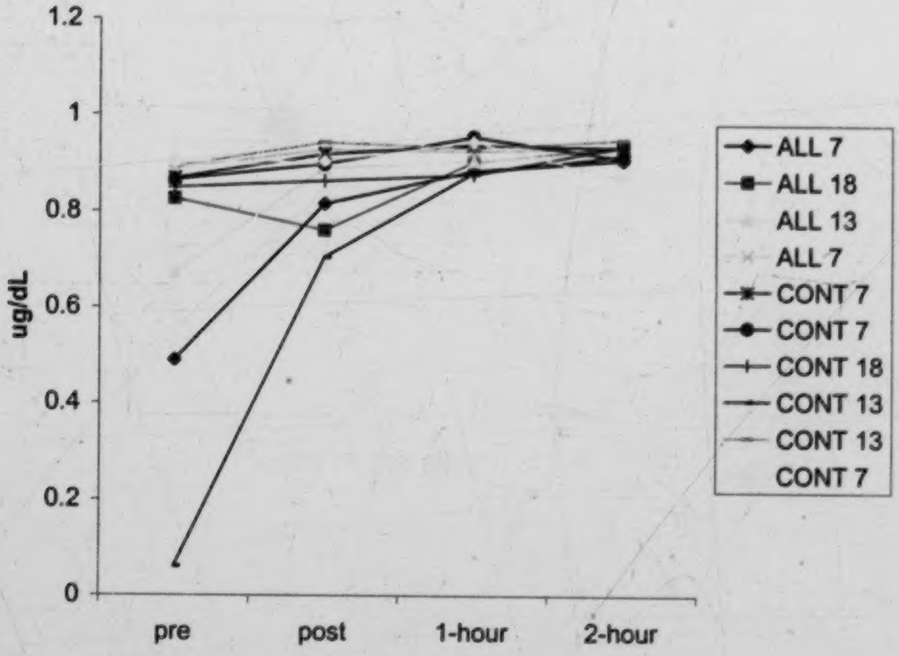

Figure IV – 10. Salivary Cortisol Response

IV – 4. ADVERSE EVENTS DURING THE STUDY

There were two adverse events during the study. One of the healthy age- and gender-matched control participants found the heart rate monitor suffocating near the end of the 30-minute submaximal exercise session on visit 2 of testing. The participant became very upset and the session was stopped immediately, approximately 4 minutes prior to finish. The participant quickly calmed down and felt better. The post-exercise blood sample was subsequently drawn and the participant finished the rest of the testing session without any further problems. The second adverse event included the ALL participant that was rescheduled due to an inability to find the antecubital vein after several attempts to insert butterfly needle, causing bruising to the participant.

V: CHAPTER FIVE

DISCUSSION

The purpose of this study was to examine the effects of acute moderate-intensity aerobic exercise on peripheral blood neutrophil concentration and function, salivary IgA, and salivary cortisol in children with acute lymphoblastic leukemia receiving maintenance treatment in comparison to healthy, age- and gender-matched control participants. This section will be discussed in the following sections: 1) Participant Characteristics, 2) Effect of Acute Bout of Exercise On Peripheral Blood Measures, 3) Effect of Acute Bout of Exercise on Salivary Measures.

V – 1. PARTICIPANT CHARACTERISTICS

V – 1a. Exercise Minutes

A notable finding of this study was the difference in the total exercise minutes per week between the ALL participants and the healthy age- and gender-matched controls. On average, the ALL participants exercised 215 minutes and the healthy participants exercised 491 minutes per week. This equates to 30 minutes of activity per day for the ALL patients and 70 minutes of exercise per day for the healthy participants. Further, the amount of strenuous exercise per week (defined in the questionnaire as heart beating rapidly, sweating) was reported to be 33 minutes in the ALL group versus 291 minutes in the healthy group. Moreover, moderate activity (not exhausting with light perspiration) was reported to be 153 minutes versus 208 minutes in the ALL and control group, respectively. Similarly, mild exercise requiring minimal effort and no perspiration was reported as 51 minutes in the pediatric oncology patients and 150 minutes in the healthy controls. Guidelines from the American Cancer Society recommend a daily minimum of 60 minutes moderate physical exercise, defined as effort equivalent of a brisk walk, engaging large muscle groups and increased heart rate and respiration.[29] Recommendations from the U.S Centres for Disease Control and Prevention (CDC) and the American College of Sports Medicine (ACSM) encourage all persons more than 6 years old to accumulate at least 30 minutes of moderate-intensity physical activity on most and preferably all days of the week.[112, 134] In older children, 20 to 30 minutes of vigorous exercise at least 3 times a week is encouraged for greater benefits.[112, 134] This could be easily achievable in the normal routine of a child's day with physical education class at school. However, for a child with cancer undergoing treatment, it may be difficult incorporating exercise in a routine that is focused predominately on treatment, recovery and returning to school. The data from this study suggest that the total daily energy expenditure of the ALL participants is reduced compared to healthy children and that this likely contributed to the reduced cardiovascular fitness of the ALL participants in this study. A number of studies in the literature also report significantly reduced energy expenditure[126, 158, 159] and decreased exercise tolerance[135] in survivors of ALL compared with healthy control participants. Although more research is needed, some data suggest that the reduction in total daily energy expenditure of ALL survivors may lead to further deconditioning.[153]

V – 1b. Peak Exercise Results

Results from the maximal graded aerobic exercise test showed a significant difference in the average duration of the graded maximal aerobic exercise test between our two groups studied. On average, the control group walked for 3.3 minutes longer on the incremental exercise test than the ALL group. There were no significant differences found between groups in maximal respiratory exchange ratios or maximal heart rates achieved, however, this is likely due to a small sample size and low power of the study. A theoretical difference was demonstrated on both variables, as the control group reached a RERmax of 1.15 and HRmax of 186 beats per minute whereas the ALL group reached 1.03 and 171 beats per minute, respectively. The maximal heart rates, respiratory exchange ratios and exercise times achieved in response to treadmill maximal incremental exercise testing by the control participants in this study are similar to that seen in previous reports in healthy children of the same age, [12, 23, 91] suggesting that the ALL participants may not have been able to push themselves to maximal exertion.

Peak oxygen update (VO_{2peak}) is considered by the World Health Organization (WHO) to be the single best indicator of aerobic physical fitness.[136] It is also a valid indicator of health status[94] and a powerful predictor of mortality in both healthy and diseased individuals.[20, 104] The present study found a significantly diminished cardiovascular fitness in the pediatric oncology patients in comparison to the healthy control participants. A significant difference was found in the relative VO_{2peak} in ml/kg/min attained during the graded maximal aerobic exercise test to volitional exhaustion between the two groups, which may be clinically meaningful. There was no significant difference in the absolute VO_{2peak} in L/min, however, a theoretical difference was demonstrated in that the control participants achieved an absolute VO_{2peak} 10.7% greater than the ALL participants. The ALL group achieved a relative VO_{2peak} of 29.1 ± 5.9 ml/kg/min compared with a VO_{2peak} of 41.2 ± 7.0 ml/kg/min in the healthy control group. These control participants, although small in number, have comparable data to that reported in the literature using incremental treadmill protocols.[12, 131, 160] The difference of 12.1 ml/kg/min would be expected to result in significantly higher risk of disease and mortality in the ALL population based on a recent prospective study of 6,213 adult men. Data from this study reported that each 1-MET decrease in exercise capacity conferred a 12% relative increase in mortality.[104] A recent meta-analysis performed by van Brussel and colleagues reported that VO_{2peak} was reduced in a total of 102 survivors of childhood ALL compared to 99 healthy control participants and found a significant reduction of 5.97 ml/kg/min.[152] The authors did not indicate the number of years post treatment for the ALL survivors. The results from the present study already show declines in physical fitness in ALL patients while on maintenance treatment. This difference is likely due to the leukemia and the side effects of its treatment. However, it is important to note that two of the four ALL patients did not achieve a maximal or peak VO_2 according to the criteria defined in Chapter III – 6a, while all of the control participants achieved a VO_{2peak}. This may account for the large differences in peak exercise results between the two groups. In addition, due to the convenience sample of healthy controls used in this study, some control participants may have been athletes, thus reporting greater exercise minutes per week as well as being more physically fit, resulting in higher VO_{2peak} results achieved.

Functional side effects in children undergoing cancer treatment have been reported in previous reports. These include an increased risk of obesity,[150] excessive weight gain due to therapy,[80] reduced cardiovascular and pulmonary function[72] and impaired muscle strength.[65] The ALL participants were on average 20 kg heavier than the control participants. According to the Centres for Disease Control and Prevention, BMI-for-age is used for children and adolescents to assess their weight.[1] The 2000 CDC growth charts for boys aged 2-20 years reveals that the BMI of the ALL group is greater than the 95% percentile, which is considered to be overweight.[1] Our findings suggest that cardiovascular conditioning is more reduced for the ALL group than for the healthy control group, which may in part be due to weight gain from the cancer treatment. Further, a study conducted by Warner and colleagues revealed that total energy expenditure and physical activity were correlated with percentage body fat, indicating that obesity in survivors of ALL may, in part, be explained by a decrease in their total daily energy expenditure as a consequence of their low physical activity levels.[158]

The possible etiological factors responsible for diminished exercise capacity in this population are many. In the present study, at the time of VO_{2peak} testing, all participants with acute lymphoblastic leukemia were receiving maintenance therapy treatment consisting of antimetabolites, methotrexate and 3 mercaptopurine. Chemotherapeutic agents have known toxicities on different organ systems and can affect the function of lung, muscle or cardiac tissue. Methotrexate is frequently used in the treatment of malignant disease and can cause pneumonitis and compromised lung function.[143, 157] Craniospinal irradiation, cyclophosphamide or bacterial lung infections during or subsequent to treatment for leukemia can reduce total lung capacity.[72] Jenney et al reported that survivors of childhood leukemia have impaired lung function and diminished exercise capacity in comparison to a matched control group.[72] The authors also attributed these decreases to irradiation and chemotherapeutic agents referred to previously. The delivery of oxygen to the working muscles may be compromised due to inadequate cardiac output mediated through organ damage caused by anthracycline drug treatment in the induction phase of treatment for high-risk patients. Subnormal cardiac function during exercise has been reported in a small group of cancer patients treated with this drug.[99, 135] The utilization of oxygen at the muscle level may be affected by a lack of oxidative capacity of muscle secondary to disuse.[100] Muscle atrophy is a common problem in this population group due to the catabolic effects of several chemotherapeutic agents such as vinicristine or corticosteroids.[65, 159] Muscle atrophy and altered muscle function are further aggravated by the catabolic effects that a sedentary lifestyle and prolonged bed rest induce on skeletal muscle tissue.[86] As a result, muscle atrophy and fatigue due to low to moderate physical tasks become self-perpetuating conditions.[158] Only physical training can break the 'vicious cycle' of sedentary habits and subsequent exercise intolerance.[86]

V – 1c. Nutrition

Immune system components are dramatically influenced by diet and nutritional status.[52, 148] The nutritional analysis via 24-hour recall showed no significant differences between the ALL group and control group except for very small differences in percent kcal from protein. The healthy control participants had a higher percent of calories in their diet from protein and monounsaturated fats. However, both groups met the current recommendations for the distribution of energy from macronutrients intake of 45-65% carbohydrate, 25-35% fat and 10-30% protein.[34] The small differences in the distribution of protein between groups did not likely alter the immune and hormonal measures taken in this study before and after exercise.

The ALL participants compared to the healthy participants had similar amounts of carbohydrate in their diet and both groups had an average of 53% of total calories from carbohydrate. In addition, participants in the study were given a standardized breakfast based on their caloric intake for their height, weight and age consisting of a protein bar and orange juice. Both groups consumed approximately 43 g of carbohydrate. Dietary fibre intake was significantly different between the groups, suggesting that the healthy control participants consumed more whole grains and/or fruits and vegetables in their diet prior to the exercise session. The macronutrient distribution was similar between both groups of participants, therefore it is likely that diet did not influence their immune responses.

V – 1d. Fasting Measures

Fasting samples of blood and saliva were taken first thing in the morning following a 10-hour fast from the previous night. All samples were taken at the same time of the morning, at a basal state, before any food was ingested or any activity was performed. There were no significant differences in the saliva measures, however a significant difference was observed in the relative lymphocyte and neutrophil percent and lymphocyte and eosinophil concentration in peripheral blood between the groups. The ALL group had a lower relative lymphocyte percent as well as lymphocyte concentration and eosinophil concentration versus the control group. These differences are likely due to the leukemia itself and the effects of the chemotherapy treatments in the ALL participants.[54, 56, 80]

V – 2. EFFECT OF ACUTE EXERCISE ON PERIPHERAL BLOOD

V – 2a. Complete Blood Count

The acute bout of exercise caused a significant increase in white blood cells, red blood cells, hemoglobin, hematocrit and lymphocyte concentration and a significant decrease in eosinophil concentration from pre to post-exercise in both groups. A significant decrease occurred during recovery from post exercise to 1-hour and post-exercise to 2-hours in the WBCs, RBCs, hemoglobin, hematocrit, relative lymphocyte percent as well as lymphocyte concentration in both groups, suggesting that the decrease in total WBC is due to a decrease in lymphocytes. Eosinophil concentration significantly decreased in both groups from post-exercise to 2-hours post exercise only. Although most immune cells increased in concentration in blood during exercise, a brief period of immunosuppression, termed the "open window" is hypothesized to occur during recovery from strenuous exercise and this may increase susceptibility to infection.[116] The depression in lymphocytes observed at post-exercise in this study may confer some susceptibility. It is important to note that the current understanding of exercise-induced changes in components of the human immune system is derived primarily from adults, and there is a paucity of data in children. A few studies[47, 118, 137] have reported similar responses to aerobic exercise between children and adults, however, closer inspection of the data would suggest that that magnitude of change in various immune cells was smaller in younger participants compared with older ones.[158] Furthermore, children tend to have a faster physiologic recovery i.e. heart rate and ventilation from exercise than do adults, thus immune cells in their systems may also recover more rapidly, thereby limiting the "open window" period.[16] This open window pattern likely explains the decreases observed in the present study for WBCs, RBCs, hemoglobin, hematocrit, and lymphocyte concentration in both groups of participants from post-exercise to 1-hour and 2-hours post exercise.

Data from the present study show that the relative percent of neutrophils, eosinophils and lymphocytes were significantly different between the two groups, likely due to the leukemia and the chemotherapy treatments for the ALL participants. However, the white blood cell response to exercise was similar between the two groups. The WBCs increased similarly in both groups (30-36%) following the acute bout of exercise. Yet, during recovery, a marked decrease in WBCs was observed in the ALL group, while this response was not found in the control group. The WBCs of the ALL participants decreased by 4% at 1-hour post exercise while the control group exhibited a 14% increase from pre-exercise levels. At 2-hours post exercise, the ALL group showed a slight increase of 6% while the control group showed an increase of 24% from pre-exercise. White blood cells consist of lymphocytes, neutrophils, monocytes and the small populations of basophils and eosinophils. The cell populations that likely accounted for the WBC difference during recovery are the neutrophils, basophils, a small proportion of eosinophils and the lymphocytes. The neutrophil concentration over the four sampling time points is discussed below in Section V – 2b. The basophil response during recovery in the ALL group was vastly different from the control group. At 1-hour post exercise, the basophil concentration in the ALL group decreased by 25% while it increased in the control group by 7% from baseline values. Similarly, at 2-hours recovery, the basophil concentration decreased by 16% in the ALL group, however it increased by 14% in the control group from pre-exercise values. The decrease in basophils at both recovery time points may account for the lower WBC response observed following exercise in the ALL group. Additionally, the eosinophil concentration at 1-hour recovery was quite different between groups. Although levels decreased in both groups from pre-exercise levels, the eosinophil concentration in the ALL group decreased by 20% more than the control group. This decrease may also contribute to the lower WBC response observed in the ALL group during recovery.

The lymphocyte concentration was also significantly different between the two groups with the ALL group exhibiting abnormal values at all the sampling time points. The acute exercise bout elicited a favourable increase in lymphocytes in both groups of participants, however a decrease to baseline values was observed by 1-hour recovery. Lymphocyte concentration remained significantly lower in the ALL group versus the healthy control group during the recovery period. Very low levels of lymphocytes or lymphocytopenia can leave patients vulnerable to life-threatening infections caused by viruses or fungi. The abnormal lymphocyte concentration is likely due to the leukemia itself and the chemotherapeutic agents given during maintenance therapy. Although the proportion of lymphocytes increased similarly in both groups throughout the sampling period, it is likely that the depressed lymphocytes observed in the ALL group account for the lower WBC counts found in these participants compared with the healthy control participants.

In addition, a different peripheral blood response was observed between the ALL group and the control group in RBCs, haemoglobin and hematocrit levels. The RBCs were not within the normal range for the ALL participants throughout the sampling time points. Further, hematocrit was abnormal throughout sampling with the exception of post-exercise. Moreover, hemoglobin values were on the lower end of the normal range for the ALL group throughout the sampling time points and were abnormal at 1-hour post exercise. These data suggest that the ALL participants may have been anemic at the time of the submaximal testing. The abnormal RBC response in the ALL participants is likely due to the chemotherapy treatments that they were receiving during the maintenance phase, which causes myelosuppression[30, 33, 35, 54, 56, 155] including anemia.[6]

V – 2b. Neutrophil Count and Function

The results from the present study show a significant difference in the neutrophil response between the two groups. Although the ALL group exhibited greater relative percentages of neutrophils and greater neutrophil concentrations than the control group over the four sampling time points, the actual neutrophil response to exercise was lower in the ALL group. The data show that immediately following exercise, the ALL participants exhibited a 26% increase in neutrophil concentration, while the controls demonstrated an increase of 47%. At 1-hour recovery, the neutrophil concentration of participants in the ALL group dropped by 3%, while it continued to increase in the control group by 33% from baseline values. At 2-hours recovery, the neutrophil concentration in the ALL group increased by 6%, whereas it increased in the control participants by 49% from pre-exercise levels. Preliminary studies from Cooper and colleagues and data from Nemet and colleagues revealed post-exercise increases in circulating neutrophils ranging from +32 to +104% in healthy and obese children during a variety of exercise formats.[39, 106] The results from this study showed that our healthy control participants responded similar to those children reported in the literature following acute exercise. However, the ALL participants in the present study demonstrated a lower ANC response to exercise compared to both the healthy controls in this study and the literature reported in healthy children. These results may be clinically meaningful as they suggest that the depressed ANC response in the ALL participants is likely due to the chemotherapeutic agents they were receiving, which are reported to cause myelosuppression[30, 33, 35, 54, 56, 155] and depletions in neutrophils. [54, 56, 80]

A Pearson correlation between absolute neutrophil count and salivary cortisol showed an inverse relationship such that as cortisol levels increased at post exercise and 1-hour post exercise, absolute neutrophil counts decreased at post-exercise and 1-hour post-exercise. This may explain the decrease in ANC observed from post to 1-hours post exercise in both groups. A subsequent increase in ANC occurred from 1-hour to 2-hours recovery in both groups, a 9% increase in the ALL participants and a 12% increase in the control participants. Interestingly, the ALL group had 38% higher ANC at pre-exercise compared with the control group, however this difference slowly tapered off following the cessation of exercise and by 2-hours recovery, there was an ANC difference of only 13% between the groups.

The initial neutrophil rise is most likely due to the response of catecholamine-induced demargination of neutrophils previously adherent to the endothelial tissue.[123] The delayed rise several hours after the cessation of exercise may be the result of cortisol-induced release of mature neutrophils from the bone marrow (discussed further in Section V – 3b) into circulation or the mobilization of neutrophils from the same source in response to chemotactic signals from tissue damaged during the exercise bout.[32]

The results from the neutrophil function assay to measure oxidative burst demonstrated no significant main effects over Time, however, a significant main effect for Group was observed at stimulation time zero across all the sampling time points from pre-exercise to 2-hours post exercise. At time zero, there is no PMA stimulation. PMA mimics bacteria, thus the presence of it causes neutrophils to become active and produce reactive oxygen species in order to kill the infectious agent. At time zero, the control participants had a significantly greater respiratory burst response measured by relative fluorescence. At pre-exercise, the control group showed 70% greater oxidative burst versus the ALL participants. Similarly, this was observed at post-exercise, 1-hour and 2-hours post exercise with the control group exhibiting 64%, 47% and 54% greater oxidative burst than the ALL group, respectively. This data may be clinically meaningful. It suggests that the ALL group had a significantly depressed neutrophil oxidative burst activity because they were immunosuppressed in comparison with the healthy control participants. The ALL group demonstrated a significantly greater number of circulating neutrophils as shown by the relative neutrophil percent and the ANC results, however, it seems that their neutrophils were not as active at the zero time as those in the control group.

The results of the neutrophil function further show that once stimulated with PMA, the neutrophils in both groups respond favourably. The ALL group revealed a 2710% increase in oxidative burst measured by relative fluorescence to stimulation at times 5 from time zero. The control group showed a 536% increase in oxidative burst to stimulation at time 5 from time zero. Similar results were observed at stimulation time 5 from pre-exercise to 2-hours post exercise in both groups. Further increases in oxidative burst were observed at time 10 and 15 in both groups over the four sampling time points. This data demonstrates that the participants with ALL respond to PMA stimulation similar to the healthy control participants. Moreover, the neutrophil oxidative burst responses to the acute bout of exercise were similar in both groups. The graphs in Figures IV –2, 3, 4 and 5 illustrate that the ALL group elicited greater neutrophil oxidative burst activity versus the controls at all three stimulation time points. This is likely due to the greater concentration of neutrophils observed at fasting and prior to exercise in the ALL participants, which resulted in greater oxidative activity when stimulated with PMA.

The ratios of neutrophils at time 5, 10 and 15 were used to compare the resting neutrophils at basal to the neutrophils activated by the PMA. The ALL participants had significantly higher ratios at all three stimulation time points, which may in part be due to the low basal oxidative activity observed. However, the ALL group demonstrated that they could respond similar to the controls when a challenge such as an infectious agent or PMA is presented, suggesting that the ALL participants did not have impaired neutrophil function when stimulated.

In reference to hypothesis one (Chapter I – 3), from pre-exercise to post exercise, absolute neutrophil count increased in both groups of participants. Neutrophil function as measured by oxidative burst was shown to be significantly different at the basal state between the groups. The ALL group had a significantly depressed neutrophil oxidative burst at time zero, likely due to the immunosuppressive effects of chemotherapy treatments. However, an increase in oxidative burst was observed in both groups from pre-exercise to post-exercise once stimulated by PMA at time 5, 10 and 15.

The above findings are important as neutrophils play a crucial role in host defence by killing or engulfment of invading microorganisms.[102] Activation of the oxidative burst contribute to the cytotoxic capacity of the neutrophil. Thus, an increase in circulating neutrophils in blood along with an increase in their oxidative capacity following acute exercise is a favourable response, especially for those pediatric patients with a compromised immune system due to chemotherapy. No study to date has looked at the effects of acute exercise on neutrophil concentration and function in pediatric cancer patients. Thus, the findings from this study may be of clinical importance for the pediatric patient during periods of low immunity throughout maintenance treatment for leukemia.

V – 3. EFFECT OF ACUTE EXERCISE ON SALIVARY MEASURES

V – 3a. Salivary IgA

The salivary IgA responses to exercise in this study did not show statistical significance between groups or over time, however the trends in the data are important to note. The graph in Figure IV – 7 shows the mean response of both groups over the four sampling time points. The graph in Figure IV – 8 shows the response of each individual participant. All participants responded similarly with the exception of two participants, one control aged 13 and one ALL participant aged 18 years. Most of the participants responded with a trend towards an increase in sIgA levels following the acute bout of exercise, which remained increased at 1-hour post exercise and then levelled off by 2-hours post exercise. The percent increase at post-exercise, 1-hour and 2-hours post from pre-exercise levels was 66%, 198% and 168%, respectively in the ALL group. The control group demonstrated a -7%, 3% and 180% change at post-exercise, 1-hour post and 2-hours post exercise from pre-exercise levels. These data are important to note as IgA levels, essentially, were maintained throughout the acute exercise bout. All participants, with the exception of two, exhibited higher than baseline values to 2-hours recovery. Thus, the third hypothesis is supported by this data (Chapter I – 3), a significant decrease in sIgA levels was not demonstrated following the exercise intervention or during the recovery period. The acute exercise bout seemed to be of an appropriate moderate intensity, was not exhaustive and appeared to avoid eliciting a significant suppression in salivary IgA. The risk of infection or upper respiratory track infections due to a decrease in sIgA in response to exercise is, therefore, minimal in this study.

There is some heterogeneity in the literature. Several studies have examined the effects of moderate intensity exercise using the treadmill or cycle ergometer in a laboratory setting and found similar results.[97,89] McDowell and colleagues indicated that running at intensities of 50-80% of VO_{2max} for durations of 15-45 minutes did not significantly change salivary IgA levels at post-exercise, 1 or 2 hours post exercise from pre-exercise levels.[97] In another study, recreational joggers ran on a treadmill for 40 minutes at 55% and 75% of VO_{2peak} equivalent to moderately intense exercise. Salivary IgA was sampled pre and post-exercise and results indicated no significant changes in IgA secretion rates in the joggers.[89] On the contrary, a study by Tharp and Barnes conducted with male members of a university swim time show significant decreases in sIgA following a 2-hour swim training exercise session.[146] However, the authors do not mention the intensity level of the swimming session, thus it is possible that the decrease in sIgA could be attributed to an exhaustive, highly intensive swim session and not a short, moderate bout of acute exercise as performed in the present study. Lastly, Blannin et al conducted a study with eighteen males performing an acute bout of exercise on the cycle ergometer at 80% or until exhaustion on day one and 55% VO_{2max} for 3 hours or to fatigue, whichever was sooner on day two.[21] Saliva samples were collected at pre-exercise, during, post-exercise and 1, 2.5, 5 and 24 hours post exercise.[21] IgA concentration was greater immediately post-exercise at both intensities, though significantly decreased at 1 and 2-hours post exercise than baseline values.[21] Again, it is important to note that the latter study examined cycling to exhaustion at two different intensities, whereas the present study looked at an acute, short bout of moderate intensity treadmill running.

The literature also reports that cortisol levels have been associated with immunosuppression.[36] Reports from Hucklebridge and colleagues have shown that increased cortisol secretion caused a decrease rate of salivary IgA secretion.[67] However, the present study found no significant correlations of salivary cortisol to IgA at any of the sampling time points. IgA levels were maintained post-exercise and during the recovery period. Thus, the increased levels of cortisol did not directly affect the secretory immune system in this study.

V – 3b. Salivary Cortisol

The salivary cortisol responses to exercise did not show statistical significance between groups or over time, however the trends in the data are important to note. An increase in salivary cortisol was demonstrated from pre-exercise to 2-hours post exercise. Salivary cortisol response over the four sampling time points was similar in both the ALL and control groups shown by the means in Figure IV – 9. Salivary cortisol increased by 18% immediately following acute exercise and continued to increase 2-hours post exercise (+29%) in the ALL group. Similarly, the control group increased by 18% from pre-exercise to post-exercise and continued to increase 1-hour post exercise (+25%), followed by a plateau to 2-hours post exercise. The graph in Figure IV – 10 shows the salivary cortisol response from pre-exercise to 2-hours post exercise in all participants in the study. The results of the present investigation support the second hypothesis (Chapter 1-3), as both groups of participants showed an increase in salivary cortisol over the four time points. The cortisol results at post-exercise and 1-hour post exercise inversely correlated with absolute neutrophil count at post-exercise and 1-hour post-exercise. This suggests that the increase in cortisol levels during the 1-hour recovery period caused a decrease in the absolute neutrophil count at the same time point, which is seen in the graph in Figure IV – 6. Since the ALL group had a greater cortisol response during the recovery period, it may explain the larger depression in neutrophil counts from post-exercise to 1-hour post exercise in comparison to the control group.

There are several factors that are known to influence the response of cortisol to exercise. These include the intensity and duration of exercise, the time of day, pre-exercise cortisol level, and whether or not food was ingested prior to exercise. Circulating cortisol concentrations are maximal in the early morning hours just before awakening, as a result of increased cortisol secretory pulse amplitude and frequency, modulated by a circadian rhythm.[74] The amplitude of cortisol secretory pulses progressively decreases throughout the day. Exercise performed immediately after food ingestion results in a blunted cortisol response to the exercise stimulus.[25, 26] This study controlled for the confounding effects of food ingestion and timing of assessments. Fasting salivary cortisol responses in both groups of participants were similar. Any responses were, therefore, likely attributed to the effects of the acute exercise bout. Further, it seems that the acute exercise bout was of appropriate intensity to spark an increase in salivary cortisol.

The literature in acute exercise and salivary cortisol has shown conflicting results. To date, only one study has been performed to determine the effects of exercise on salivary cortisol in children.[41] Salivary cortisol concentration increased by as much as 81% above resting level in ten healthy male children following a 30-minute bout of cycling at 70% of VO_{2peak}.[41] Our study showed a salivary cortisol increase of as much as

29% in the ALL group and 25% in the control group from pre-exercise levels. Similar to the present study, Dimitriou and colleagues examined the acute responses of salivary cortisol in 14 male competitive swimmers following 5 x 400 m front crawl at 85% of their seasonal best time.[44] The exercise caused a significant 21% increase from baseline values pre-exercise.[44]

The present study may support the McCarthy and Dale model in that increases in plasma cortisol act after 1 to 2-hours following acute exercise to release newly differentiated neutrophils from the bone marrow.[95] Increases in salivary cortisol from pre-exercise were observed in both groups in the present study during recovery of exercise. Further, a correlation between salivary cortisol and absolute neutrophil count was observed from post-exercise to 1-hour post exercise. However, the increase in salivary cortisol was associated with a decrease in neutrophil count from post to 1-hour post exercise. It is possible that the increase in salivary cortisol is responsible for the delayed increase of neutrophils into circulation in both the ALL and control groups, as many studies have reported strong correlations between saliva cortisol and serum unbound cortisol.[41, 109, 151] Correlations, ranging from 0.60 to 0.93 have been observed at rest, during and following exercise.[41, 109] The response of salivary cortisol to exercise was observed to be similar to serum cortisol response in both timing and pattern.[109] It is also possible that a significant main effect was not observed in this study due to the small sample size of the study. Further research is required in order to support the model and subsequent work by McCarthy and colleagues.[96]

VI: CHAPTER SIX

SUMMARY AND CONCLUSIONS

VI – 1. SUMMARY

The purpose of this study was to determine the effects of acute exercise on neutrophil count and function, salivary cortisol and IgA levels in children with acute lymphoblastic leukemia receiving maintenance treatment and to ascertain if these patients respond differently than healthy, age- and gender-matched control participants.

Ten participants participated in the study, 4 ALL participants and 6 healthy matched control participants. All participants were screened to ensure all inclusion criteria were met prior to participating in the study. All 10 participants completed the salivary measures, nine provided blood samples and complete blood count measures, and seven had evaluable neutrophil function measures.

Appropriate descriptive statistics were used to characterize the participants and inferential statistics were used to determine if any significant differences existed in the characteristics of participants between the groups. Independent samples t-tests were performed to analyze the nutrition recall, fasting blood measures and neutrophil ratio results between the groups. A two-way repeated measures analysis of variance was used to assess the effect of the acute bout of exercise over the four sampling time points (pre-exercise, post-exercise, 1-hour post exercise, 2-hours post exercise) on complete blood count measures, absolute neutrophil count, neutrophil function and salivary measures, cortisol and IgA, between the groups. Pearson correlations were used to determine any correlations between cortisol and the following immune variables: salivary IgA, neutrophil concentration and relative neutrophil percent. Lastly, dependent samples t-tests were performed on all variables shown to be significant over time to determine at what sampling time points change occurred. All analyses employed the significance level of 0.100.

The nutritional intake 24 hours prior to the exercise session was analyzed in both groups to control for any confounding effects of diet on the immune response to acute exercise. The 24 hour nutrition recall suggested that the estimated intake of macronutrient was similar between the groups and that the diet consumed in the 24 hour pre-exercise period was not likely to contribute to any differences in immune response observed.

A significant difference in the cardiovascular fitness levels was found between the two groups. The ALL participants had a significantly reduced VO_{2peak} attained during the graded maximal aerobic exercise test to volitional exhaustion, which is consistent with the current literature. Another notable finding is the difference in exercise minutes between the groups. On average, the ALL group participated in 276 less minutes of exercise per week than the control group.

A significant increase in absolute neutrophil count from pre-exercise to post-exercise was found within each group. Neutrophil function as measured by oxidative burst was shown to be significantly different at the basal state between the groups with the ALL participants showing a significantly depressed neutrophil oxidative burst at time zero, likely due to the immunosuppressive effects of chemotherapy treatments. However, an increase in oxidative burst was observed in both groups from pre-exercise to post-exercise once stimulated by PMA at time 5, 10 and 15. Thus, the first hypothesis was supported by these results. The second hypothesis was supported as a trend towards an increase in salivary cortisol from pre-exercise to 2-hours post exercise was observed in both groups. It is possible that the increase in salivary cortisol demonstrated an increase in the number of neutrophils into circulation at 2-hours post exercise. A slight increase in salivary IgA was observed following the acute exercise bout and levels maintained above baseline during the recovery period. Thus, the third hypothesis was supported by this result and the exercise session was believed to be of appropriate intensity to elicit an IgA response in both groups of participants. Finally, the salivary cortisol response to the acute bout of exercise was observed to be similar between groups. Neutrophil function, following the exercise bout, was observed to be similar in both groups, however, only after stimulation. In addition, abnormal complete blood counts including a significantly depressed white blood cell count and lymphocyte concentration, depressed red blood cell count, hemoglobin, hematocrit and eosinophil concentration following exercise were observed in the ALL group while the control group showed all differentials within normal range. These results suggest support for the final hypothesis, that a different immune response was observed in the ALL participants in comparison to the healthy matched controls due to the immunosuppressive chemotherapy treatments.

VI – 2. STUDY STRENGTHS

The present study included a control group using participants matched for age and gender with the acute lymphoblastic leukemia patients. This experimental model allowed for comparison in relevant ways such as maturity level and physical fitness. The timing of assessments was standardized and controlled for all participants. The submaximal exercise bout with blood and saliva sampling was held at 9 a.m. for all participants to control for circadian effects of immune variables being measured. The multiple time point analysis of immune system function allowed for examination of recovery period following exercise. All research personnel were trained and experienced in the exercise testing, blood sampling, assay methodology and nutritional analysis. The strengths of this study provide confidence that the results were due to treatment effects and not extraneous factors.

VI – 3. LIMITATIONS

The data collected was limited to children and adolescents receiving maintenance treatment for acute lymphoblastic leukemia. The results were limited by the reliability of sampling time points and by the reliability of the project co-ordinator in calculating and providing the appropriate intensity of physical exercise. The study was designed to determine the effect of acute exercise on specific immune variables and the results provided information regarding the effect of one single moderately intense aerobic exercise session at a single intensity and duration. The effects of a training program were not examined in this study. In addition, the following factors limited the study: no female participants, a wide age range, missing data, data obtained only 2 hours post-exercise, other immune parameters not assessed and convenience sample of control participants. It is important to note that three participants in the control group were recruited from a convenience sample of children of staff or friends of staff in the Faculty of Physical Education at the University of Alberta. These control participants are likely to be more active and physically fit children and/or young athletes that may not exemplify everyday physical activity patterns of other normal and healthy children. Lastly, the study was limited by a very small, non-randomly selected sample size.

VI – 3a. Sample Size

Statistical significance was not achieved in many of the variables examines. The failure to achieve statistical significance could be attributed to the small sample size. A larger sample would have detected smaller differences. Thus, there is the potential that due to the modest sample size, the findings of the present study reflect a Type II error (find no significant difference when a difference actually does exist).

VI – 3b. Measurement

The results of the salivary assays and neutrophil function assay were limited by the reliability of the person to perform, measure and follow the protocol of the assays precisely and accurately. To ensure accuracy of the measurements, strict procedures were followed for both assays and training was provided on the assay techniques prior to conducting the assay on the blood and saliva samples for the study. In addition, the results of the study are limited to the specific assays and methods utilized and interpretation of the findings is based on the indicators used in this study.

Primary errors associated with conducting the assays are adding the correct and accurate amount of solutions, incubation time and proper centrifuge method. Utilizing one person and being comfortable to conduct all measurements and perform assays minimized these errors.

VI – 4. CLINICAL SIGNIFICANCE

A clearer comprehension of the acute effects of exercise on the developing immune system may have important clinical implications for children with or recovering from immune-related diseases such as cancer. Physical exercise is increasingly being recognized as an essential component of healthy growth and development, not merely as play. Exercise has the potential to facilitate optimal conditioning and function in a range of physical and psychological processes including cardiovascular and respiratory function, fatigue, mood, behaviour, social activity and self-esteem.[128] These beneficial effects have been reported for a variety of child populations,[57] however exercise may be of greater benefit for those children surviving cancer due to the debilitating effects of the treatments and the disease itself. Children diagnosed with acute lymphoblastic leukemia now have up to 80% chance of survival with the recent improvements in detection and treatment.[128] As survival rates continue to increase, the effects of treatments are becoming more important. Survivors are at risk of suffering a wide range of secondary problems including obesity, cardiovascular disease, fatigue, decreased quality of life and immune impairments, which exercise is shown to improve.

This investigation is significant given the potential implications for the prescription of moderate-to-vigorous intensity exercise in the pediatric oncology population. The results of this study suggest that an acute bout of moderate intensity exercise does not elicit any significant negative response in the immune system of children and adolescents receiving maintenance treatment for acute lymphoblastic leukemia, but rather promotes a positive, favourable and similar response to that of healthy children of their own age and gender. Thus, further research to determine the effects of chronic exercise in this population is warranted. In the future, exercise may potentially be regarded as a safe and beneficial activity for children with acute lymphoblastic leukemia and perhaps can be prescribed by pediatric oncologists and nurses as supportive therapy during treatment.

VI – 5. SUGGESTIONS FOR FUTURE RESEARCH

This study has highlighted the need for several areas of future research and consideration:

1. Further research with a larger sample size is required to examine immune function in response to acute exercise in patients with ALL and other pediatric cancers during treatment.
2. Further study is needed examining the immune response to chronic exercise in children receiving treatment for ALL, thereby supporting and establishing physical exercise as supportive therapy.
3. Future research should examine the intensity, duration and frequency of exercise that can elicit a positive, favourable immune response versus a suppressive response in an already immunosuppressed population
4. As immune response occurs over time, research into the recovery period following exercise greater than 2-hours post exercise should be studied.
5. Future studies should consider incorporating other immune variables as outcome measurements in order to fully evaluate and observe immune responses to acute exercise in this population.
6. Research is needed to observe the effect of an exercise program on the quality of life, immune response and physical fitness of ALL patients.

VI – 6. CONCLUSIONS

Impaired physical fitness leads to early fatigue during physical activities and can severely deteriorate the quality of life of ALL survivors suggesting there is a need for these children to engage in regular physical activities. Exercise physiologists could assist pediatric oncologists in prescribing exercise to help attenuate cancer-related fatigue, improve physical fitness, quality of life and the immune system for these children receiving treatment for cancer. The findings of this study suggest that an acute bout of 30 minutes of moderate intensity exercise does not cause an adverse neutrophil, salivary cortisol or IgA response in acute lymphoblastic leukemia patients receiving maintenance treatment. The findings also suggest that efforts towards prescribing exercise should be implemented early to help prevent declines in physical functioning such as cardiovascular fitness and weight gain due to aggressive chemotherapy treatments.

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APPENDICES

APPENDIX A. DEFINITIONS

Remission – the absence of clinical signs of disease.

Prophylaxis – drug treatment (chemotherapy) given to prevent future occurrences of disease.

Intrathecal – drugs administered into the cerebrospinal fluid (CSF) bathing the spinal cord and brain.

Relapse – to regress after partial recovery from illness.

Allogenic - being genetically different although belonging to or obtained from the same species.

Anemia - deficiency in the oxygen-carrying component of the blood, measured in unit volume concentrations of hemoglobin, red blood cell volume, or red blood cell number.

Thrombocytopenia - an abnormal decrease in the number of platelets in circulatory blood.

Neutropenia - an abnormal decrease in the number of neutrophils in the blood.

Osteopenia – decreased calcification or density of bone, reduced bone mass.

Chemotaxis – movement of a cell along a concentration gradient towards or away from chemical stimulus.

Phagocytosis - the engulfing and ingestion of bacteria or foreign bodies by phagocytes.

Immunosuppressive – drugs and/or radiation that lower the body's normal immune response.

Myelosuppression - suppression of bone marrow activity resulting in a reduction of the number of platelets, red and white blood cells found in circulation.

Sepsis – a toxic condition resulting from the presence of pathogenic organisms, spread of bacteria or other toxins in the blood or tissues.

APPENDIX B: ETHICS APPROVAL

APPENDIX C: ADMINISTRATIVE APPROVAL

APPENDIX D. PARTICIPANT PACKAGE

COVER LETTER PATIENT

<date>

Dear Parent:

We are writing to ask if your child will take part in a research study exploring the potential effects of physical exercise on immune function in children and adolescents undergoing maintenance therapy for acute lymphoblastic leukemia. The study protocol has been approved by the Health Research Ethics Board and the Northern Alberta Pediatric Program. We have not released, and will not release your child's personal information, and all of the information that you provide will be held in strict confidence.

In our previous research we found that physical exercise can have a number of significant beneficial effects for cancer patients both during and after cancer therapy. However, most of the research to date has been conducted in breast, colorectal, prostate, or bone marrow transplant patients. No study to date has examined the potential role of exercise on immune function in children and adolescents with acute lymphoblastic leukemia undergoing maintenance therapy. It is unknown whether or not patients should be exercising, and it is unknown if a different response to exercise exists between patients and healthy individuals. The information gained from this study will be used to evaluate the potential role of physical exercise during therapy and it's role as an intervention to improve immunity and quality of life in young acute lymphoblastic leukemia patients.

The first part of the study involves reading the enclosed consent forms. Please read and sign one copy of the Consent Form, Assent Form, and Blood Draw Consent Form. A second copy of each is enclosed for your records. Next, please complete the questionnaire with your child. The questionnaire should take approximately 20-30 minutes to complete. There are no right or wrong answers and all we ask is that you and your child provide responses that are honest and accurate as possible. You may choose to leave any question in the questionnaire unanswered. The second part of the study involves two exercise tests. The first is a maximal exercise test in which your child will jog on a treadmill until he or she is fatigued and can no longer continue. This entire session (warm up, cool down etc.) will last approximately half an hour. The second exercise test will include a walk/jog for 30 minutes with repeated saliva and blood sampling. This entire session will last approximately four hours. Visits will be scheduled at the Cross Cancer Institute and the University of Alberta Behavioural Medicine Fitness Centre.

Your child's participation in this study is absolutely voluntary. If you choose not to participate please disregard this, or any future information you may receive about our study. However, it is only through voluntary participation in research projects that we increase our knowledge about issues that are important to acute lymphoblastic leukemia patients.

COVER LETTER FRIEND

<date>

Dear Parent:

We are writing to ask if your child will take part in a research study exploring the potential effects of physical exercise on immune function in children and adolescents undergoing maintenance therapy for acute lymphoblastic leukemia. Your child's friend currently undergoing treatment thought you might be interested in participating in this study. The study protocol has been approved by the Health Research Ethics Board and the Northern Alberta Pediatric Program. We have not released, and will not release your child's personal information, and all of the information that you provide will be held in strict confidence.

In our previous research we found that physical exercise can have a number of significant beneficial effects for cancer patients both during and after cancer therapy. However, most of the research to date has been conducted in breast, colorectal, prostate, or bone marrow transplant patients. No study to date has examined the potential role of exercise on immune function in children and adolescents with acute lymphoblastic leukemia undergoing maintenance therapy. It is unknown whether or not patients should be exercising and it is unknown if a different response to exercise exists between patients and healthy individuals. Thus, your child will act as a healthy control for comparison in this study. The information gained from this study will be used to evaluate the potential role of physical exercise during therapy and it's role as an intervention to improve immune function and quality of life in young acute lymphoblastic leukemia patients.

The first part of the study involves reading the enclosed consent forms. Please read and sign one copy of the Consent From, Assent Form, and Blood Draw Consent Form. A second copy of each is enclosed for your records. Next, please complete the questionnaire with your child. The questionnaire should take approximately 20-30 minutes to complete. There are no right or wrong answers and all we ask is that you and your child provide responses that are honest and accurate as possible. You may choose to leave any question in the questionnaire unanswered. The second part of the study involves two exercise tests. The first is a maximal exercise test in which your child will jog on a treadmill until he or she is fatigued and can no longer continue. This entire session (warm up, cool down etc.) will last approximately half an hour. The second exercise test will include a walk/jog for 30 minutes with repeated saliva and blood sampling. This entire session will last approximately four hours. Visits will be scheduled at the Cross Cancer Institute and the University of Alberta Behavioural Medicine Fitness Centre.

Your child's participation in this study is absolutely voluntary. If you choose not to participate please disregard this, or any future information you may receive about our

INFORMATION LETTER

Title of Project: Effects of an acute bout of exercise on mucosal IgA, cortisol and neutrophil count and function in individuals undergoing maintenance therapy for acute lymphoblastic leukemia.

Principal Investigator(s): Dr. Kerry Courneya, Ph.D.
Professor, Faculty of Physical Education and Recreation

Co-Investigator(s):

Dr. Paul Grundy	Director, Pediatric Oncology, Stollery Children's Hospital
Dr. Catherine Field	Associate Professor, Agriculture, Food & Nutritional Science
Dr. Gordon Bell	Professor, Physical Education & Recreation
Aliya Ladha	M.Sc Student, Faculty of Physical Education & Recreation

Purpose: This study will determine the effects of physical exercise on immune function in individuals undergoing maintenance treatment for acute lymphoblastic leukemia. In addition, this study will aim to discover if acute exercise affects the immune system differently in patients versus healthy individuals.

Background: Cancer treatments cause numerous physiological effects, one of which is suppression of the immune system. This may lead to infections causing further morbidity and even cancer recurrence. Interventions designed to improve immune status in young patients are therefore warranted. Physical exercise has been shown to enhance various immune variables in healthy populations, however, limited evidence exists for cancer patients, especially children. No study to date has examined the potential role of exercise on immune function in children and adolescents with acute lymphoblastic leukemia undergoing maintenance therapy. It is unknown whether or not patients should be exercising and it is unknown if a different response to exercise exists between patients and healthy individuals.

Procedures: Once you agree to take part, you and your child will be given a questionnaire package to complete including a past exercise questionnaire, maturation stage questionnaire, and quality of life questionnaire. A maximal exercise test on a treadmill will be scheduled for your child at the Cross Cancer Institute as an initial screening test. This involves your child jogging until he or she is fatigued and can no longer continue.

A second visit will be scheduled for a second exercise test on a treadmill and blood and saliva sampling. This exercise test will involve a walk/jog at a moderate intensity determined by the maximal exercise test on day one. The actual exercise session will last 30 minutes with an additional 5-10 minutes of warm-up and cool-down exercises before and afterwards. On the evening prior to this second visit, your child will be asked to fast for 12 hours overnight. In the morning, a butterfly catheter will be inserted in the arm in order to obtain blood samples, which will remain until all sampling is completed. A fasted blood sample will be taken first thing by the nurse, which will be followed by a breakfast for the child and then the exercise session. Blood collection and saliva collection will occur again just prior to the exercise session, immediately following, 1-hour and 2-hours post-exercise. One final questionnaire to assess 24-hour dietary intake will be completed with a registered dietician present during this second visit.

The total number of samples: 5 blood samples (1 tablespoon each time), and 4 saliva samples. The total time commitment is as follows: questionnaire 20-30 minutes, maximal aerobic exercise test 1 hour, submaximal aerobic exercise test, saliva and blood sampling 4 hours.

Benefits: Your child may benefit by finding his or her personal health fitness information. The child will be provided with physical fitness scores according to the norms of his/her age group. There may be no direct benefit to the child as a result of participating in this study.
However, the information from this study may help us understand whether exercise is an effective intervention for immune function in patients undergoing maintenance treatment for lymphoblastic leukemia.

Risks: Aerobic fitness will be measured during an exercise test until the child says he or she cannot continue. The exercise intensity is quite light at the beginning of the test and becomes more difficult every minute. The actual test will last approximately 10 minutes with an additional 5-10 minutes of warm-up and cool-down exercises before and afterwards. During the test, expired gases will be collected using a special breathing apparatus. Heart rate will be monitored continuously with an ECG and/or a heart rate monitor.

The aerobic fitness test requires maximal effort in order to go to exhaustion. There may be some health risk with this type of exercises. During and following the test, it is possible to experience symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and very rare cases (0.5 per 10,000 in testing facilities such as exercise laboratories, hospitals and physicians' offices), heart rhythm disturbances or heart attack. While serious risk to healthy participants is highly unlikely, they must be acknowledged, and participants willingly assume the risks associated with very hard exercise. The exercise test will be performed by qualified personnel trained to handle identifiable risks and emergencies, and have certification in CPR. In addition, a physician will be present (if using the ECG) to interpret the recordings to ensure the safety of the patient during the maximal exercise test.

During the occasions on which your child has blood drawn, he or she may have a slight degree of discomfort. The insertion of the catheter for blood sampling will result in a puncture of the skin, which may lead to a bruise or infection, but this is normal and only temporary. These risks will be minimized, as a trained and qualified nurse will perform sampling.

CONSENT FORM

Title of Project: Effects of an acute bout of exercise on mucosal IgA, cortisol, and neutrophil count and function in individuals undergoing maintenance therapy for acute lymphoblastic leukemia.

Principal Investigator: Dr. Kerry Courneya, Ph.D.
Professor, Faculty of Physical Education and Recreation

Co-Investigators:

Dr. Paul Grundy	Director, Pediatrics, Stollery Children's Hospital
Dr. Catherine Field	Associate Professor, Agriculture, Food & Nutritional Science
Dr. Gordon Bell	Professor, Physical Education & Recreation
Aliya Ladha	M.Sc Student, Faculty of Physical Education & Recreation

Do you understand that your child has been asked to take part in a research study? Yes No

Have you and your child read and received a copy of the Information Sheet? Yes No

Do you understand the benefits and risks involved for you child in taking part in this research study? Yes No

Have you had a chance to ask questions about what your child will be doing? Yes No

Do you understand that your child can refuse to participate or withdraw from the study at any time? You and your child do not have to provide a reason. Yes No

Do you understand the issue of confidentiality and who will have access to your child's records? Yes No

This study was explained to me by: ______________________

I agree for my child to take part in this study.

Signature of Parent or Guardian

Date

Signature of Child

Printed Name

Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

Signature of Investigator

Date

CHILD ASSENT FORM

Title of Project: Effects of an acute bout of exercise on mucosal IgA, cortisol, and neutrophil count and function in individuals undergoing maintenance therapy for acute lymphoblastic leukemia.

Principal Investigator: Dr. Kerry Courneya, Ph.D.
Professor, Faculty of Physical Education and Recreation

Co-Investigators:

Dr. Paul Grundy	Director, Pediatrics, Stollery Children's Hospital
Dr. Catherine Field	Associate Professor, Agriculture, Food & Nutritional Science
Dr. Gordon Bell	Professor, Faculty of Physical Education & Recreation
Aliya Ladha	M.Sc Student, Faculty of Physical Education & Recreation

You have acute lymphoblastic leukemia and have to exercise. We would like you to try jogging on a treadmill for half an hour. Eight other children or teenagers with acute lymphoblastic leukemia and eight healthy children or teenagers will take part in this study.

What will you have to do? If you and your parents agree to take part, we will ask you to fill out a survey about how much you exercise, how mature you are, and how you feel. We will ask you to come to our lab 2 times for exercise tests. The first one, you will jog on a low level and every minute, the level of hill will increase until you are too tired to jog anymore. This test will take about half an hour. For the second test, you and a friend will jog on the treadmill together for 30 minutes and give 5 blood samples and spit in a tube five times. Each blood sample will measure about one tablespoons. The total time for the second test will be four hours. You will also fill out a survey about what food you ate the day before.

Will it help? You may feel better while you are exercising, but you may not.

Will it hurt? The blood test may hurt since the nurse has to insert a butterfly with a needle. Your muscles may be sore after jogging and you may feel tired. You must tell your mom or dad or your doctor about anything you think is different.

Can you quit? You don't have to take part in the study at all, and you can quit at any time. No one will be mad at you if you decide you don't want to do this, or if you decide to stop part way through. You should tell the project director that you want to quit.

Who will know? No one except your parents, the doctor, and the research team will know you're taking part in the study unless you want to tell them. Your name and your chart won't be seen by anyone except the people involved in the study.

Your signature: We would like you to sign this form to show that you agree to take part. Your mom or dad will be asked to sign another form agreeing for you to take part in the study.

and let her know that you do not want the Behavioural Medicine Laboratory Research Team at the U of A to use your child's sample(s), and they will no longer be used for research. Otherwise, the sample(s) may be kept until they are used up, or until the Behavioural Medicine Laboratory Research Team at the U of A decide to destroy. Samples will be destroyed by a strong, pressurized, steam-heated vessel and will be discarded.

Your child's sample(s) will be used only for research and will not be sold. The research done with your child's sample(s) may help to develop new products in the future, but you or your child will not get paid.

CONFIDENTIALITY

All bloods drawn for this study will be kept in a freezer designed to store biological specimens. Only registered lab technicians will be able to access your child's blood out of the freezer.

Data collected from participants in this study will be kept in a secure location in the Behavioural Medicine Laboratory of the University of Alberta. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the following:

- Health Research Ethics Board (who oversees the ethical conduct of this study)

Each person looking at your child's records will follow the relevant policies and procedures that control these actions. However, your child will not be identified by name in any information released or in information published as a result of this study.

Please read each sentence below and think about each choice. After reading each sentence, circle "yes" or "no". If you have any questions, please talk to your doctor or nurse. Remember, no matter what you decide to do about the storage and use of your child's sample(s), your health care will not be affected in any way.

By signing this form, you are agreeing that:

1. Your child's blood sample may be kept for use in future research to learn about or treat cancer.

   YES NO

2. Your child's samples(s) may be used for research about other health problems.

   YES NO

3. You wish your child's study doctor (or someone he or she chooses) to contact you in the future if further research on these samples is to be performed.

YES NO

I will get to keep a copy of this consent for information and for future reference.

(PRINT NAMES CLEARLY)

Name of Parent/Guardian	Signature of Parent/Guardian	Date
Name of Child	Signature of Child	
Name of Investigator	Signature of Investigator	Date

The information below is needed to help understand the characteristics of all the children participating in the study. For this reason it is very important information. All information is held in strict confidence and your child's name will NOT appear on any public documents. Please answer the following questions based on the present status of your child.

1. Age of the child: ______

2. Gender: (please circle)

   M F

3. *Current grade at school:* ______

4. Is your child attending school regularly at the present time? (please circle)

   Yes No Attending occasionally

Please continue on other side

This section is important in understanding past exercise behaviour. We would like your child to recall his/her average weekly exercise during maintenance therapy for acute lymphoblastic leukemia. Considering a typical week (7 days) how many times on average, did your child perform the following kinds of exercise?

When answering these questions please:

- **Only count exercise sessions that lasted 20 minutes or longer in duration.**
- Only count exercise that was done during **OWN FREE TIME** (DO NOT include gym class at school, competitive school teams or organized/competitive sports outside of school).
- Note that the main difference between the three categories is the **intensity** of the exercise
- Please write the average frequency on the first line and the average duration on the second

Times Per Week Average Duration

a. STRENUOUS EXERCISE (HEART BEATS RAPIDLY, SWEATING) ____________ ________

(e.g., running, swimming, hockey, soccer game, rollerblading, laser tag)

b. MODERATE EXERCISE (NOT EXHAUSTING, LIGHT PERSPIRATION) ____________ ________

(e.g., fast walking baseball, shooting hoops, easy bicycling, badminton, leisure skating)

c. MILD EXERCISE (MINIMAL EFFORT, NO PERSPIRATION) ____________ ________

(e.g., easy walking, bowling, frisbee)

Please continue on other side

This next section is important in understanding stage of maturation. We would like you to read each of the stages and indicate which one best describe your child's phase of puberty.

I. Girls

Tanner Stage	Stage of develop	Pubic Hair	Breasts
Stage 1	Early adolescence (10-13 years)	Preadolescent	Preadolescent
Stage 2		Sparse, straight	small mound
Stage 3	Middle adolescence (12-14 years)	Dark, curl	bigger; no contour separation
Stage 4		Coarse, curly, abundant	Secondary mound of areola
Stage 5	Late Adolescence (14-17 years)	Triangle; medial thigh	nipple projects; areola part of breast

I. Boys

Tanner Stage	Stage of develop.	Pubic Hair	Penis	Testes
Stage 1	Early adolescence (10.5-14 years)	None	Preadolescent	Pre-adolescent
Stage 2		Scanty	Slight increase	larger
Stage 3	Middle adolescence (12.5-15 years)	Darker, curls	Longer	larger
Stage 4		adult, coarse, curly	Larger	scrotum dark
Stage 5	Late adolescence (14-16 years)	adult - thighs	Adult	adult

My child is at Tanner Stage _____.

Please continue on other side

The next section is important in assessing food intake. This section will be filled out during day two of exercise testing with a registered dietician.

What did you eat for late night snack yesterday?	For Each Food Group Mark the number servings
______________________________	☆ △ ✸
______________________________	○ □

What did you have for dinner yesterday?

______________________________	☆ △ ✸
______________________________	○ □

Afternoon Snack yesterday?

______________________________	☆ △ ✸
______________________________	○ □

Lunch yesterday?

______________________________	☆ △ ✸
______________________________	○ □

Morning snack yesterday?

______________________________	☆ △ ✸
______________________________	○ □

Please continue on other side

Breakfast yesterday?

______________________________ ☆ △ ✹

______________________________ ○ □

TOTALS: Grain products 5-12 ☆ Vegetables & Fruit 5-10 △
Milk Products 3-4 ○ Meats & Alternatives 2-3 □

Please continue on other side

Is there anything else you or your child would like to tell us? Please feel free to use as much space as you need to make any comments regarding your diagnosis or treatment, this study, or exercise.

Thank you very much for your participation in this research project. Please bring the completed questionnaire along with a signed copy of the informed consent and blood draw consent form with you to your first exercise test.

APPENDIX E. MODIFIED BALKE PROTOCOL

Level	Speed (mph)	Grade (%)	Time (mins)
1	3.5	2	1
2	3.5	4	1
3	3.5	6	1
4	3.5	8	1
5	3.5	10	1
6	3.5	12	1
7	3.5	14	1

APPENDIX F. EXERCISE TESTING FORM

Exercise Testing Form

Demographic Information

DATE: ____________

Name:	Phone #
Address:	
Age:	Date of Birth:

Medical History/Exercise Contraindications

Assessments

Mass (kg):__________ Height (cm): __________ BMI (kg/m^2) __________

Resting HR (bpm): __________ Resting BP (mmHg): __________

Pre-exercise BP (mmHg): ________________

Post-exercise BP (mmHg): ________________

Exercise Capacity Assessment

Time (Min)	Grade (%)	Speed (mph)	Heart Rate (bpm)	RPE
1	0	3.5		
2	2	3.5		
3	4	3.5		
4	6	3.5		
5	8	3.5		
6	10	3.5		
7	12	3.5		
8	14	3.5		
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

2 min recovery HR:________________ 2 min recovery BP: ________________

5 min recovery HR: ________________ 5 min recovery BP: ________________

Comments:

Reason Max test was stopped:

Cart Specifications

Pre Test Cal 0_2:________________________ Pre Test Cal $C0_2$: ____________________

Post Test Cal 0_2:________________________ Post Test Cal $C0_2$: ___________________

Correction Needed? Yes / No

APPENDIX G. SUBMAXIMAL EXERCISE TESTING FORM

Submaximal Exercise Testing Form

Demographic Information **DATE:** ______________

Name:	ID #

HR Vslope (AT) = ______________ HR 70% peak VO_2 = ______________

Fasting Blood __________ Fasting Saliva _____________

Breakfast

Juice _________ mL Powerbar ___________ calories

Pre-exercise blood ___________ Pre-exercise saliva __________

Post-exercise blood __________ Post-exercise saliva __________

1-hour recovery blood ________ 1-hour recovery saliva ________

FILL OUT 24-HOUR DIETARY RECALL

2-hour recovery blood _________ 2-hour recovery saliva ________

GIVE T-SHIRTS TO PARTICIPANTS

Submaximal Exercise Bout

Stage	Speed (mph)	Heart Rate (bpm)	BP (mmHg)	RPE
Warm-up				
5 mins JOG			▨	
10 min JOG			▨	
15 mins WALK			▨	
20 mins WALK			▨	
25 mins JOG			▨	
30 mins JOG			▨	
Cool down				

APPENDIX H. PARTICIPANT TRACKING SHEET

Effects of an acute bout of exercise on mucosal IgA, cortisol, and neutrophil count and function in individuals undergoing maintenance therapy for acute lymphoblastic leukemia

Participant Tracking Sheet

Name: ______________________ Address: ______________________

Phone #: ______________________ ______________________

DOB: ______________________ ______________________

ID: ________

Medical Records:

Oncologist: ______________________________

Month and Year of diagnosis: ______________________

Type of therapy : ______________________________

Venous Access Device______________________________

Height (cm): ______________________ DATE: ______________________

Weight (kg): ______________________

Received from Participant:

- ☐ Assent: ______________________________
- ☐ Informed Consent: ______________________
- ☐ Blood Consent: ______________________
- ☐ Baseline Questionnaire: ______________________

Dates to be scheduled:

- ☐ Fitness test: ______________________
- ☐ Submaximal exercise bout: ______________________

Participant Tracking Sheet (Control)

Friend/Control for: ________________

Name: __________________________ Address: ____________________

Phone #: ________________________ ____________________

DOB: ___________________________ ____________________

ID: ________

Received from Participant:

☐ Assent: _________________________________

☐ Informed Consent: _________________________

☐ Blood Consent: ___________________________

☐ Baseline Questionnaire: ____________________

Dates to be scheduled:

☐ Fitness test: _____________________

Submaximal exercise bout: _____________________